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Studies on barley yellow dwarf virus in west-central Morocco

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Studies on barley yellow dwarf virus in west-central Morocco

by

Mohamed El Yamani

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DEDICATION

To my wife Zahra, my son Adnane, and
daughter Mouna for their
patience and sacrifice.

To my parents
To my brothers
To my sisters
To all my relatives, friends, and instructors.

INTRODUCTION

Barley yellow dwarf (BYD) is a virus disease of cereals and grasses caused by barley yellow dwarf virus (BYDV). It was first recognized and named by Oswald and Houston (62) in 1951 in California. Earlier reports and notes by several scientists concerning widespread red leaf of oats were summarized by Bruehl (5). Since 1951, research on this disease has multiplied and other names have been suggested (6, 7, 48, 63, 77, 97) but the original one is too well established to change.

The virus is now classified as the type member of the luteovirus group which includes several members with similar properties (58). Some of these properties are: isometric particles about 25 nm in diameter, a single stranded positive sense RNA genome of Mr 2.0×10^6, a single coat protein of Mr $22\text{-}24 \times 10^3$, a sedimentation coefficient of 115 to 118 S, and transmission by aphids in a persistent manner. Also, studies conducted by Miller et al. (59, 60) have provided valuable information on the sequence and organization of the genomic RNA and coat protein of the virus.

The virus is restricted to the phloem in the plant host and is strongly immunogenic when injected into warm-blooded animals. Diagnostic symptoms include leaf yellowing of cereals and grasses. There is, however, symptom variation which is dependent upon crop cultivar, growth stage at time of infection, virus strain, and environmental conditions (e.g., photoperiod, light intensity and temperature).

Detailed descriptions of disease symptoms on the major cereals have been provided by previous studies (5, 8, 64, 104). In general, the virus

interferes with the translocation of carbohydrates within the plant by partially blocking the phloem. Among the other effects of virus infection are severe stunting of infected plants, inhibition of the root system and reduction of yield components. Several color changes are induced by BYDV infection in major cereal crops. In barley, a bright yellow discoloration begins at the leaf tip and margins and moves rapidly downward towards the stem. The mid-vein area is the last to change color. In oats, leaves turn a reddish or purple color in the same manner as in barley. Additional symptoms in oats include plant stiffening and head blasting. In wheat, rye and triticale, the discoloration varies from yellow to purple. Leaf serration occurs in wheat which is severely infected. Rye, however, expresses slight symptoms. Maize shows purpling and yellowing of leaves following virus infection (61, 94). Rice leaves become yellow to orange following inoculation with the virus (2). In general, the incubation period for symptom expression is 7 to 20 days depending on the plant host, although, in general, incubation is shorter and disease symptoms are more severe in oats, followed by barley and then wheat.

Many other grasses are hosts of BYDV in addition to the cereals previously discussed. Bruehl (5) summarized the known host range of the virus prior to 1961 which included plant species in California, New York, Washington and Great Britain. Ninety-seven species from 36 genera within the Gramineae were susceptible. Later reports included those of Slykhuis (89), who listed about a hundred susceptible graminaceous species, as well as those documented by other investigators (22, 49, 93). The large host range included susceptible and symptomless species within both the annual

and perennial grasses. No dicotyledonous species, however, are known to be infected by BYDV. Although, BYDV is restricted to the Gramineae, the magnitude of the host range and the diversity of the host species enable the virus to survive under diverse conditions as shown by various reports (16, 21, 22, 37, 38, 39, 49, 51, 70).

The virus particles are limited to the phloem of infected plants (34, 35, 36, 44). Infected plants are characterized by an increase in respiration, a decrease in photosynthesis, a partial or total inhibition of the translocation of carbohydrates, and a severe reduction in yield (43, 45).

Only one instance of seed transmission of BYDV has been reported (95). These results were not confirmed by Eweida et al. (20) although virus was detected in the inner tissues of oat seed by enzyme-linked immunosorbent assay. The principal vectors of BYDV are aphids. Recent work by Jess and Mowat (46) suggested successful transmission of BYDV by larvae of the fruit fly, Oscinella frit (L.). A complete listing of aphid vectors of the virus has been recently published by A'Brook (1) and Jedlinski (41) in which they described 23 and 18 aphid species, respectively. The principal aphid species vectoring the virus throughout the world are Metopolophium dirhodum (Walker), Rhopalosiphum padi (L.), R. maidis (Fitch), Schizaphis graminum (Rond.) and Sitobion (Macrosiphum) avenae (Fab.) (8). Sekkat (86), in a study from 1979 to 1987, reported R. padi (L.), R. maidis (Fitch), Sitobion avenae (Fab.), S. fragariae (Walk.), Schizaphis graminum (Rond.), Diuraphis noxia (Mordvilko ex Kurdjumov) and Sipha (Rungsia) maydis (Passerini) on wheat in the Meknes area of Morocco. The same author, also,

reported other aphid species on several diverse gramineous hosts occurring in the same area.

Most researchers agree that BYDV is not transovarially transmitted to progeny although aphid nymphs may be just as efficient as adults in transmitting the virus (31, 68). Further, the relative importance to virus epidemiology of the different aphid species as vectors of BYDV in a country or even a region is dependent upon the prevalence of different virus isolates as demonstrated by Gill (29, 30) and Rochow (75, 76). Research has demonstrated the existence of great variation within the BYDV group. Rochow (74, 76) defined BYDV strains based on virus-vector relationships. Subsequent work confirmed these findings (29, 30, 83).

At present, BYDV strains are designated by the initial letters of their principal vector or vectors. The established ones are RPV-transmitted specifically by Rhopalosiphum padi (L.); RMV-transmitted specifically by Rhopalosiphum maidis (F.); MAV-transmitted specifically by Macrosiphum (Sitobion) avenae (FAB.); SGV-transmitted specifically by Schizaphis graminum (Rond.) and PAV-transmitted mainly by R. padi (L.) and S. avenae (Fab.) and rarely by R. maidis (F.) and S. graminum (Rond.). The transmission pattern seems to be relatively consistent where BYDV research has been done (19, 23, 69, 90).

Historically, these strains could also be differentiated serologically (66, 81). The relationship between vector specificity and strain specificity does not appear to be absolute, however. In China, Zhou and Zhang (105) differentiated among isolates, termed GPV, which were transmitted nonspecifically by S. graminum (Rond.) and R. padi (L.) but

which did not react with the antisera prepared against the American isolates MAV, PAV, RPV and RMV; GAV, transmitted nonspecifically by S. graminum (Rond.) and S. avenae (Fab.) which are serologically related to MAV and PAV variants, PAGV, transmitted nonspecifically by R. padi (L.), R. maidis (F.) and S. avenae (Fab.) which are serologically similar to the PAV isolate, and finally the RMV variants which resembled their American counterparts serologically and in aphid transmission. These findings and others (53) suggest the presence of luteoviruses serologically distinct from those originally described by Rochow and Gill in North America. In fact, Lister and Sward (53) reported the occurrence in Victoria, Australia, of BYDV isolates serologically similar to MAV strain, originally described by Rochow (76), but more similar to the PAV strain in vector relationship. The same authors also noticed some changes in serological behavior, using monoclonal antibodies, of an MAV isolate maintained in culture at Purdue although its efficient transmission by S. avenae was well maintained. In their studies on serological relationships among the various strains, Rochow and Carmichael (81) found that PAV and MAV types were serologically related and distinct from RPV and RMV which were also related. SGV appeared to be related to the PAV strain. Similar subgroups were described by Gill and Chong (35, 36) using comparisons of ultrastructural changes induced in oat tissues by the isolates studied.

Apparent virus-vector specificity can appear to break down, however, under specific conditions such as determined by the age of the infected source plant at the time of virus acquisition (26) or, as determined in mixed infections, in which a phenomenon, termed dependent transmission by

Rochow (78, 79) and Rochow and Gill (82) occurs. In the latter case, the hypothesis is that, during simultaneous synthesis of two virus isolates in the host cell, transcapsidation of one strain's RNA with the other's coat protein occurs. This provides opportunity for transmission by the otherwise nonvector aphid. This is possible because virus-vector specificity appears to be dependent on specificity of receptor sites in the accessory salivary glands of the aphid for the viral capsid protein (27, 28).

Specific aphid transmission has been the basis for BYDV-strain identification and is still reliable in surveying for strains of the virus. However, the transmission tests are laborious and time-consuming and other analytical techniques have been devised and adapted for use in BYD research. Two such techniques using specific antisera are serologically specific electron microscopy (SSEM) and enzyme-linked immunosorbent assay (ELISA). SSEM has been successfully used by Paliwal (65, 67) to detect the virus in plant tissue and in aphid vectors. At present, this technique is quite common and widely used (14, 15, 71). In 1979, ELISA was adapted for the detection of BYD-luteoviruses in plant tissue (52) and in aphid vectors (12). The advantages of ELISA include sensitivity, rapidity and the large number of samples that may be assayed. These made ELISA the assay technique of choice among those currently described. Rochow (80), and Rochow et al. (84), in extensive comparative studies of ELISA with aphid transmission, concluded that in 80% of the cases the two techniques agreed. In another 18% of the isolates, ELISA was more informative. Consequently, ELISA has become a routine test in many laboratories and many variants of

the test exist (99, 100, 101, 102). Other assay methods which may have enhanced sensitivity, such as nucleic acid hybridization, are becoming increasingly common (13, 54, 103).

The BYD disease is known to be ubiquitous as it has been reported from various parts of the world (5, 77, 88, 89). During the BYD workshops held in Mexico in 1983 and in Italy in 1987, more than 20 situation reports were delivered on the disease which literally covered the 5 continents. The economic importance, in terms of losses caused by this virus, is very much time and location dependent. Many attempts have been made to estimate the magnitude of these losses. Bruehl (5) summarized the losses found in the United States prior to 1961. He reported losses as high as 50% in oats, 30% in wheat and 20% in barley in South Dakota in 1959. Schaller and Qualset (85) reported that, prior to the release of varieties tolerant to BYDV, yield losses in the United States could attain an average of 19% in barley. Losses as high as 60% could be measured if individual test differences were considered. These data were obtained in yield loss trials using the barley lines CM 67 and California Mariout isogenic for the Yd_2 gene for virus tolerance. Important losses have been similarly reported from Canada (32, 33), France (4) and Australia (91, 92). In addition to yield loss, BYD can affect the quality of the grain (33) and reduce pasture productivity (40, 50, 96).

Losses caused by BYD disease can be minimized as a result of avoiding infection through crop management techniques such as sowing date, seeding rate, crop rotation, use of fertilizers, and elimination of grasses (4, 5, 9, 10, 24, 47, 61); by killing the aphid vectors (3, 4, 5, 25, 69) or by

breeding for host resistance to the virus. The latter option, although difficult to accomplish, appears to be the best choice because of environmental hazards that may arise as a result of insecticide application to control the vectors and the lack of flexibility, in numerous places, to implement crop management techniques. Fortunately, sources of resistance are available in some Ethiopian barleys. The resistance in this species was found to be controlled by one incompletely dominant gene, Yd_2 (73, 87). The situation is quite different in oats and wheat, however, where resistance has been detected but is not controlled by a major gene as in barley. However, resistance levels higher than those presently existing in these species may be achieved by selection (42, 72, 98).

In Morocco, agriculture is by far the leading economic activity and provides income for about 65% of the Moroccan population (55). Cereal cropping represents 80% of the area devoted to agriculture in the country. Barley occupies 39% of the hectarage covered by cereals, followed by wheat with 31%. Other cereals, such as rice, sorghum, maize, millet, oats and rye, although considered secondary in comparison with barley and wheat, are also grown in Morocco. National average cereal yields are still very low and vary between 800 kg per hectare for maize and 1310 kg per hectare for wheat (57). In the geographical area covered in this study (Fig. 1), cereal yields are even lower. Wheat yields are 950 to 1000 kg per hectare in the Chaouia, 400 to 500 kg per hectare in the Rhamna-Haouz, 600 to 800 kg per hectare in the Chiadma and 750 to 800 kg per hectare in the Doukkala-Abda areas. Respective barley yields for the same areas are 1300, 750, 900 and 900 kg per hectare (56). The main reasons for these low

yields are, in addition to the genetic potential of the varieties used, the low and erratic annual rainfall (200-400 mm), Hessian fly damage, and plant diseases including BYD. In fact, visual estimations of the incidence of BYDV-like symptoms done by J. Burleigh (Professor, Institute of Agronomy and Veterinary Sciences, Hassan II, Rabat, Morocco, personal communication) were 75% in the bread wheat var. Nesma 149 and 90% in the durum wheat var. Kyperounda. Later, El Yamani (17) and El Yamani and Hill (18) established that the etiology of these symptoms were caused by BYDV. This marked the beginning of research on this important problem in the country. With this background, a commitment was made to investigate the importance of BYD disease in the west-central region of Morocco. This thesis will emphasize the following aspects:

*Identification of strains and their relative distribution in nature using various assay techniques.

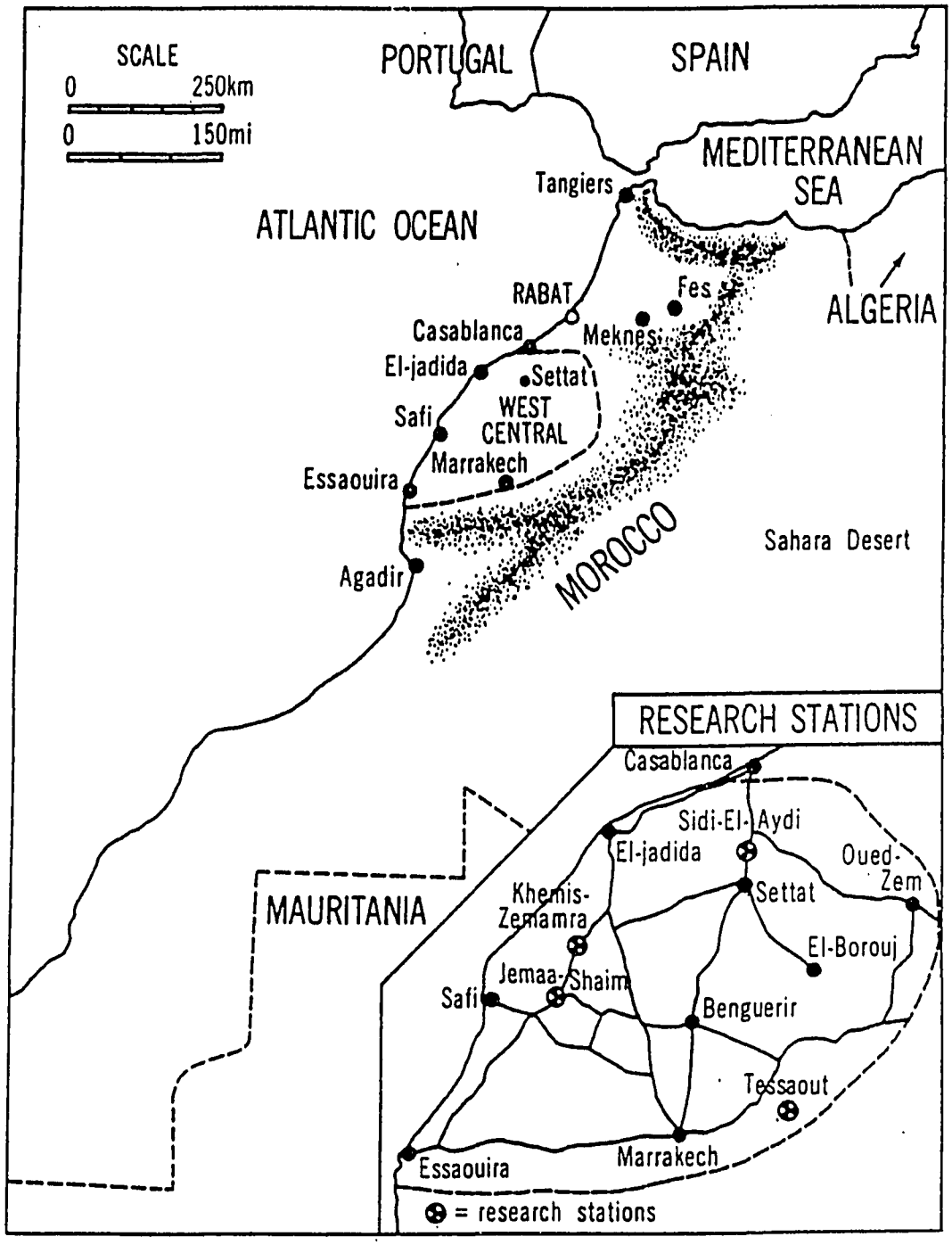
*Determination of potential vectors among the aphid species in the area.

*Establishment of a list of hosts susceptible to Moroccan isolates of BYDV.

*Investigation of cereal crop losses due to the disease in the aridoculture region.

*Investigation of resistance to the virus in Morocco by screening barley, wheat and oat germplasm.

Figure 1. Map of Morocco indicating the west-central area and the experiment stations located in the area. The latter include the sites of the crop loss assessment trials in this study.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Explanation of Dissertation Format

This dissertation is presented in the alternate format approved by the Graduate College. It includes a general introduction with a critical review of the literature on the subject and four sections representing papers to be submitted to scientific journals, namely,

Paper I. Characterization, grass hosts, and epidemiology of barley yellow dwarf virus isolates in west-central Morocco,

Paper II. Grass aphid species in relation to the transmission of barley yellow dwarf virus isolates in west-central Morocco,

Paper III. Purification and serology of a Moroccan isolate of barley yellow dwarf virus,

Paper IV. Crop loss assessment and germplasm screening for resistance to barley yellow dwarf virus in west-central Morocco.

It also includes a summary-discussion of the entire subject, an appendix section, and acknowledgments.

The entire research was done by the senior author, Mohamed El Yamani, who is also the doctoral candidate under the supervision of the coauthor of these manuscripts.

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SECTION I: CHARACTERIZATION, GRASS HOSTS, AND EPIDEMIOLOGY OF BARLEY YELLOW DWARF VIRUS ISOLATES IN WEST-CENTRAL MOROCCO

Characterization, grass hosts, and epidemiology of barley yellow dwarf virus isolates in west-central Morocco

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ABSTRACT

The PAV, MAV, and RPV strains of barley yellow dwarf virus have been identified in west-central Morocco. The PAV strain occurred most commonly (56%) followed by the MAV (35%) and the RPV (9%) strains. The disease can reach epiphytotic conditions as illustrated by the 1986-87 growing season. Disease incidence was greatest during spring, and the virus and vectors oversummered in maize and cereal volunteer plants. The aphid vector Rhopalosiphum padi was constantly present. Other aphid species were generally initially detected in February and could be detected as late as May. Evaluation of 16 grass species collected in the field and 17 species planted from seed showed that 16 and 15 of those tested were susceptible, respectively. Some of the susceptible gasses remained symptomless after inoculation.

INTRODUCTION

Barley yellow dwarf (BYD) disease occurs world wide and is caused by a persistently transmitted virus called barley yellow dwarf virus (BYDV), a member of the luteovirus group (2, 24). The virus has been the subject of many studies to define criteria for strain identification. Allen (1) differentiated seven virus strains on the basis of symptoms on differential plant species, mainly barley and oats. Later, Rochow (23) defined 4 virus strains on the basis of aphid transmission specificity. Subsequently, Rochow and Carmichael (25) and Paliwal (20) found that serological techniques to differentiate the strains correlated with aphid transmission.

Other aspects of the disease and its causal agent have been studied in different parts of the world. In the Mediterranean area, however, research on BYD has been limited to the southern European countries. Few investigations have been made in North African or Middle Eastern countries bordering the Mediterranean Sea (7, 17). The objective of this research was to further define the serological and biological characteristics of BYDV strains, virus incidence and disease severity in cereals, and host range of BYDV in the major cereal-growing area of west-central Morocco.

MATERIALS AND METHODS

Survey methods

Fields of wheat, barley, oat and maize were surveyed for BYDV during the 1985-1986, 1986-1987, and 1987-1988 growing seasons in the semi-arid areas of the Chaouia and Abda regions of Morocco. Stops were made approximately every 10 km along a designated route. Selected fields were entered at a corner and single leaves of 50 plants were selected at random proceeding in a diagonal configuration. Leaves from each field were combined and samples of five leaves each were processed for testing by the enzyme-linked immunosorbent assay (ELISA). Disease incidence (I) was determined for each field by using the formula, $I(\%) = (1-Q^{1/N}) \times 100$ where Q = proportion of leaf batches not infected with the virus, and N = number of leaves per batch (18). Fields in which the disease occurred in large areas were reported separately. Disease severity was reported using the scale of Schaller and Qualset (28), which ranges in severity from zero to nine with nine being the most severe.

Aphid transmission experiments

Aphid transmission of BYDV was performed according to Rochow (23). Four aphid species, *Rhopalosiphum padi* L., *R. maidis* F., *Sitobion (Macrosiphum) avenae* F., and *Schizaphis graminum* Rond. were maintained as virus-free colonies. Colonies were started from nymphs newly born on filter paper and transferred onto healthy seedlings of oats, *Avena sativa* L. 'Clintland' 64'. Species were maintained in separate 90 x 90 x 90 cm cages in a growth room (22 C and 16-hr photoperiod). Leaves from plant samples, usually with virus symptoms, were cut into pieces, 2 to 5 cm long,

and divided into four groups of 6-8 pieces each, which were then placed into containers filled 1 cm deep with moist sand and covered with cheesecloth. Fifty aphids of each of the four species were placed separately in the containers for an acquisition access period of at least 48 hours at 20 C with a 16-hr photoperiod. Aphids (5 per plant) were then transferred onto healthy Clintland '64' oat seedlings in the 2-leaf stage. The remaining leaf fragments were stored frozen at -25 C or -80 C (15) for later use in ELISA. Aphid-infested oat seedlings were caged under lights for an inoculation access period of at least 5 days. The plants were then sprayed with an insecticide (Methomyl, 25% a.i. at 1.5 g/L of water) and incubated in the glasshouse for at least 4 weeks for symptom development. ELISA was performed on samples from these plants to confirm virus infection. Another cycle of aphid transmission, termed recurrent transmission by Rochow (23), was conducted on several plant samples. The virus source plants used for acquisition access in the recurrent transmission experiments, consisted of the symptomatic aphid-inoculated plants from the original transmission experiments. At the end of the test, usually 4 to 5 weeks, the inoculated plants were evaluated visually for symptoms and serologically by ELISA.

ELISA

The double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (4, 6, 10, 15, 25) was performed by using Immulon 1 or 2 polystyrene plates (Dynatech Laboratories, Inc., Alexandria, VA). Capture antibody (diluted in coating buffer, 0.05 M sodium carbonate buffer, pH 9.6, containing 0.02% NaN_3) was added to each well (100 μl/well). The unused outer wells were

filled with distilled water. Plates were incubated at 30 to 37 C for 4 hr and washed 3 times (4 min each) with wash buffer (PBS, phosphate buffered saline, 0.01 M, pH 7.4, containing 0.05% Tween 20). Three hundred μl per well of blocking buffer (0.01 M PBS, containing 1% bovine serum albumin [BSA] and 0.05% Tween 20) were added, and the plate was incubated for 90 min at 25-30 C. Plates were washed as described and 100 μl of the test sample was added to the wells. After incubation for 12-14 hr at 4 C, and washing as previously described, 100 μl per well of conjugated antibodies diluted to their optimal concentrations [determined by calculation of P/N ratios (13)] with conjugate buffer (0.01 M PBS, pH 7.4, containing 0.05% Tween 20, 2% polyvinylpyrrolidone, MW 40,000, and 0.2% BSA) or with healthy Clintland '64' oat sap was added, and plates were incubated for 4 hr at 30 to 37 C. After washing, 100 μl of the enzyme substrate (1 mg/ml p-nitrophenyl-phosphate in 10% diethanolamine, pH 9.8, containing 0.01 % Mg $Cl_2.6H_2O$ and 0.02% NaN_3) was added. Plates were incubated 30 to 60 min at 37 C in the dark, and 50 μl per well of 3 M NaOH was added. Absorbance of reaction products was measured at 410 nm with an ELISA plate Minireader II (Dynatech Laboratories, Inc., Chantilly, VA) coupled to a Star SG-10/15 printer. All samples were run at least in duplicate and included buffer and healthy sap controls. Readings three standard deviations higher than the mean of the healthy sap were considered as positive.

Test samples were prepared by placing leaf pieces in plastic bags lined with a single layer of cheesecloth and grinding them in 0.1 M phosphate buffer, pH 7.4 (1:2, w/v), with an externally applied ball bearing pressure plate attached to a stirring motor (Bodine Electric Co.,

Chicago, IL). After dilution of the sample to 1:6 (w/v) with PBS-T-PVP (wash buffer containing 2% polyvinylpyrrolidone 40,000 M.W), the samples were used immediately or stored at -25 C or lower (16) until use. Extracts were occasionally centrifuged at 8000 g for 10 min before use.

Seven different antisera were routinely used in the ELISA (Table 1). Antiserum B consisted of polyclonal (PC) antibodies raised against the European B strain of BYDV (similar to the nonspecific PAV strain "sensu" Rochow). Antiserum F consisted of monoclonal (MC) immunoglobulin M (IgM) prepared against the European "F" strain (similar to the Sitobion avenae-specific MAV strain "sensu" Rochow). The other MCs were immunoglobulin G (IgG) prepared and characterized by R. Diaco at Iowa State University (6; unpublished results). The IgG's were purified from ascites fluid by affinity chromatography of the mixture on protein A Sepharose CL-4B (Pharmacia) as described (6). MC MAV-3B10 was used as a universal capture for MC's MAV-7F6, MAV-2B12, MAV-6G7, and PAV-3A11, referred to subsequently as 7F6, 2B12, 6G7, and 3A11. These MC's were conjugated with alkaline phosphatase as described (13, 15, 32). Homologous capture and enzyme-conjugated antibodies were used for B and F antisera. Concentrations of IgG were determined by $E_{280}^{0.1\%} = 1.4$. MC's 3B10 and 6G7, known to have higher background reactions, were diluted in the presence of healthy oat sap prepared at a buffer:healthy sap ratio of 2:1. The resulting solution was incubated for 30 min at 30-37 C and then centrifuged at 2040 g for 5 min before use.

The MAV- and PAV-infected material, used as positive controls, was kindly supplied by R. M. Lister (Purdue Univ., West Lafayette, IN) as dried

leaves of infected Clintland '64' oats.

Field studies

To investigate how BYDV and its vectors survive through the summer and the timing of virus spread in relation to cropping, plastic pots (16- cm diameter containing 10 to 20 two-week-old oat seedlings) were placed periodically at four sites (two pots per site) in west-central Morocco (Fig. 1). The bait plants were maintained one month at each site before they were brought back to the laboratory for examination. At the end of the month, plants were caged and then examined in the laboratory for presence of virus symptoms and aphid species. Plants were then recaged for another month to allow for further development of virus symptoms and reproduction of aphid species. This procedure was found necessary because, on many occasions, the few aphids present at the time of harvest were not readily discernible at the first examination. At the end of about 2 months, plants were reexamined, and presence of virus symptoms and vector species were recorded. Plant tissue was then harvested and stored at -25 C or -80 C until used in ELISA. This experiment was conducted for two growing seasons from December 1986 to June 1988. The sites chosen to conduct the experiment represent diverse climatic conditions in the investigated area (Fig. 1).

Host range

The virus host range study included two groups of plants. In the first group, seeds of several grass species were kindly supplied by A. Arif (Forage section, National Institute for Agronomic Research, Settat, Morocco). Plants, started from seeds in the greenhouse, were inoculated

with a nonspecific PAV strain of BYDV identified and maintained in our facilities. Inoculation with R. padi was as described. Plants were sprayed with insecticide, observed for symptom development, and assayed with ELISA by using the PAV antisera as described. The second group of plants consisted of symptomatic as well as symptomless grass species collected during field surveys and on other occasions. Plant samples were stored at -25 C or lower until assayed by ELISA. All available antisera were used to detect all possible virus strains. In addition, three grass species, Paspalum dilatatum Poir, from the Tessaout area, Pennisetum villosum R. Br., from Dar Bouazza, and Stenotaphrum secundatum (Walt.) Kuntze, from Settat, were collected, transplanted into pots, and challenged with the PAV strain as described.

RESULTS

Aphid transmission and ELISA

Initial attempts to study the correlation between ELISA and aphid transmission specificity for identification of BYDV-strains (Table 2) showed that 26 of 28 (93%) isolates studied contained only the nonspecific PAV strain. One sample was doubly infected with the PAV and MAV (*S. avenae*-specific) strains and, another sample, with the PAV and RPV (*R. padi*-specific) strains. The optimization of ELISA provided a reasonably low background compared the homologous reaction. Aphid colonies remained virus-free as demonstrated by the controls. In all transmission tests, *R. padi* was the principal vector in both single and double infections. Transmission rates by *R. maidis* and *S. graminium* were also quite high. Results of ELISA and those of aphid transmission from original as well as recurrent tests (ca. 100) correlated to a level of 80%. Of the remaining 20%, ELISA was more informative for 15% (data not shown). Therefore, ELISA was used to test the majority of samples collected during field surveys.

Prevalence and incidence of BYDV

Results of field surveys (Table 3) demonstrated that the nonspecific PAV strain was most frequent, followed by the MAV strain, and with the RPV strain, the least common. The presence of the three strains, either alone or in combination, was detected in 100% of the samples, with the PAV strain representing 56%, MAV strain 35%, and RPV only 9%. All the strains were identified in small grains either as single infections or in combination.

Comparison of results from ELISA of the Moroccan isolates with the American and European antisera is shown in Table 4. The chi-square (X^2)

test of independence of the reactions of F antiserum versus 2B12 and of B antiserum versus 3A11 (Appendix I) was highly significant (P = 0.01). This suggests rejection of the independence hypothesis of the reactions and implies that Moroccan isolates of BYDV reacted differently depending on the origin of the antisera used in the tests.

Incidence and severity of BYDV in west-central Morocco

The heaviest incidence of BYD disease was observed in the 1985-86 and 1986-87 growing seasons (Table 5). In 1988, there were numerous fields with less than 10% disease incidence. Virus incidence in expanded areas of severely damaged plants was very common in 1986-87. In general, the relative incidence of BYDV in barley fields was greater than that for wheat fields. Disease incidence was the highest for all crops during the 1987 growing season (Fig. 2), but, there was a general trend toward maximum disease incidence during the months of March and April. Infection of maize occurred in all three seasons, but incidence was the highest in 1986. The data also demonstrate the role of wheat, barley, and oat volunteers as potential overseason reservoirs during the months of July to September (Fig. 2).

Severity ratings in the cereal growing area over two growing seasons demonstrate that the highest scores (≥4) were recorded for barley, followed by durum and bread wheats (Table 6). The disease was also more severe in 1987 as compared with 1988.

Results obtained by monitoring survival of the virus-vector complex by use of oat bait plants indicate that, in general, R. padi was detected throughout the year. Therefore, it may be the most active virus vector.

However, there was also another period during the year, usually during the growing season from February to April, when other species were detected. Virus isolates detected in the bait plants over the 19-month duration of the survey were PAV-100%, MAV-84 %, and RPV-47%. Seven different aphid species, including the Russian wheat aphid (Diuraphis noxia Mordw.), were also detected on the bait plants during the experiment (Table 7).

Host range of BYDV in Morocco

All 16 grass species collected during the virus surveys contained at least one of the three virus strains (Table 8). Multiple strain infection was quite common, suggesting potential for virus reservoirs and intermediate hosts. Seven of these grasses are perennials. Data from the 17 grass species started from seed, all of which were perennials except Stipa retorta Cav., demonstrated that 10 of these species were susceptible, five were symptomless and only two were immune; namely, Bouteloua curtipendula and Eragrostis intermedia. The symptomless carriers were two Agropyron, one Bromus, one Eragrostis, and one Stenotaphrum species (Table 9).

DISCUSSION

Previous studies on BYDV strain identification in Morocco (7, 17), showed the presence of various virus strains. This study has confirmed and extended those results. Previous studies in Morocco used only either aphid transmission or ELISA for strain identification but did not use both techniques simultaneously. The correlation of ELISA with aphid transmission specificity was 80%, which was similar to the 83% obtained by Rochow et al. (27) in a comparative study of the two techniques as applied to BYDV strains in North America.

The high rate of PAV transmission by R. maidis and S. graminum obtained in this study has also been reported from other areas in the Mediterranean basin (17), especially in Syria, where rates as high as 30% have been reported. However, consistent PAV-like transmission by R. padi and S. avenae has also been established (23, 25). Our data demonstrating predominance of the PAV-like strain agrees with other reports from the Mediterranean area (2, 7, 11, 17, 19). Further, the predominance of the PAV isolates and their very efficient vector, R. padi, in the area may constitute a threat to cereal production in Morocco, especially since these isolates are the most virulent reported (23). It is also possible that the widespread distribution of this strain and its aphid vector may allow for transmission and spread of the other viral strains for which R. padi is not a regular vector (e.g., MAV) through dependent transmission (26).

Moroccan PAV-like isolates were more readily detected by the European antisera than by antisera prepared against American isolates. Specifically, the B antiserum was four times more sensitive than the 3A11

antiserum. The converse was true for the Moroccan MAV-isolates. The 2B12 antiserum was at least twice as sensitive for detection of Moroccan MAV isolates than the F antiserum (Table 4, Appendix I). These results suggest epitopic variation exists among isolates of the BYDV strains. The PAV strain was serologically more similar to that from Europe, and the MAV strain was more similar to that from North America. This suggests the PAV isolates may have originated in Europe. Although the hypothesis needs further investigation, it is partly supported by the abundance of PAV isolates reported from Europe (2, 11, 19) and possible vector migration as suggested by wind-speed (Appendix II) and direction (Appendix III) data from Morocco (National Meteorological Center, Casablanca, Morocco). Indeed, synthesized wind-direction data for periods ranging from 8 to 34 years at eight different meteorologic stations across Morocco showed that the predominant wind direction varied between North, West, and Southwest, with speed averages reaching 6 m/s in Tetouan, a northern station, and 22 m/s in the southernmost Laayoune station (Appendices II and III). Although the origin of the Moroccan BYDV isolates may be different, data in Table 7 suggest that the virus and at least R. padi are permanently present in Morocco. However, this is not in contradiction with what stated above since the inoculum and vectors may be endogenous as well as exogenous during the peak periods of virus and vector multiplication as previously reported in Indiana (5) and eastern Canada (21). Also, constantly changing wind directions in Morocco from north to west to southwest during the winter and spring may permit widespread dispersion of the vectors within the country. The other aphid species collected on the bait plants, M.

dirhodum Wlk. and D. noxia Mordw., expand the list of potential virus vectors in the country. The status of these two species in relation to their BYDV transmission ability is discussed elsewhere (8).

Incidence of BYDV

Virus incidence in the entire lower Mediterranean basin in 1986-1987 was highest in Morocco and Tunisia (17). Disease incidence was relatively low in 1988. Disease severity was generally highest in barley followed by that in durum and bread wheat. Severity scores ranging from 1 to 9 were registered for barleys as well as for wheats. These scores suggest potential for considerable loss because scores of 4 to 5 or higher result in appreciable crop damage (28). Those data demonstrate a necessity, in Morocco, for further effort in breeding for BYDV resistance, especially in barley.

A comparison of our findings concerning host susceptibility of grass species with those obtained elsewhere demonstrates conflicting results (Table 10). This may be due to several factors, including the virus isolate used, the vector implicated, the technique and environmental conditions of study, and the source of the grass species used in the study. Conflicting results have been reported for the same species at the same location (e.g., Agropyron cristatum, A. trichophorum, Bromus inermis, and Dactylis glomerata). A previous study in Morocco of 12 grass species separated them into susceptible, symptomless, and immune species (7). The present study comprised 18 locally grown grasses and 15 grass species introduced from seed. Seven of the 18 local grass species and 5 of the 15 introduced species have not been tested elsewhere, and no comparison was

possible for them. All of the local species tested were susceptible. The 15 introduced species contained nine with visible symptoms, four symptomless carriers, and two immune species. These data now provide information regarding potential hosts of the virus in the area. Among the native grasses of interest to the epidemiology of the virus are the perennials Arundo donax, Cynodon dactylon, Paspalum dilatatum, Pennisetum villosum, and Stenotaphrum secundatum, which are very prevalent in certain areas of the country. Precautions are also necessary to avoid introduction of new susceptible host species to enrich Moroccan pastures. According to our results, only Bouteloua curtipendula and Eragrostis lehmaniana tested-virus negative. The remainder of the species were susceptible, although symptoms were not always evident. These species could provide reservoirs for virus and vector populations if cropped. However, vector preference studies on these different grasses might prove interesting because some "force-feeding" of the viruliferous aphids on these hosts was necessary in these studies.

The existence of irrigated and nonirrigated cropping in Morocco, different crop associations (e.g., oat or wheat and vetch for forages), traditional inefficient crop management allowing for more grain loss and volunteer host plants, and numerous wild grass hosts result in an agroecosystem favoring the maintenance of the virus and vectors in the country. Further, prevalent mild weather (Appendix IV) favors persistence of the BYDV complex throughout the year. The existence of irrigated maize in the area may also act as a bridging host where summer conditions preclude survival of other host plants. The potentially important role of

maize in sustaining virus epidemics is illustrated by data in Fig. 2. Incidence of BYDV in maize in summer 1986 was significant. The virus could then be transmitted to volunteer cereal plants during the fall months of October and November to originate the virus epiphytotic of the 1987 cropping season. The role of maize as an intermediate and oversummering host of the virus and its vectors has been recognized in the United States (5, 30), in China (31), and in various Mediterranean countries (2, 11, 19). Further, temperature conditions of 10-12 C necessary to initiate vector flights (e.g., R. padi, [2]) are routinely present during November through January in Morocco. However, additional work is needed to clarify the effect of other environmental factors and crop management techniques on the virus-vector complex in Morocco.

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Table 1. Properties of antisera used to identify BYDV

Antiserum	Origin[a]	Type[b]	Known specificity to the BYDV strains			Antibody dilution or concentration (μg/ml) used in the study	
			MAV[c]	PAV[c]	RPV[c]	Coating globulins	Conjugated globulins
B	Europe	PC	--	++	--	1/500	1/800
F	Europe	MC	++	--	--	1/400	1/600
MAV 3B10	USA	MC	++	++	++	1 - 4	-----
MAV 2B12	USA	MC	++	--	--	----	4.00
MAV 7F6	USA	MC	++	--	++	----	0.25
MAV 6G7	USA	MC	--	--	++	----	0.03
PAV 3A11	USA	MC	--	++	--	----	4.00

[a]Antisera B and F were acquired from BIOREBA (Basel, Switzerland) as kits prepared against the European BYDV strains. The remainder were produced and characterized by R. Diaco at Iowa State University (6, and unpublished results).

[b]PC and MC indicate polyclonal or monoclonal antibody, respectively.

[c]MAV is the Sitobion avenae-specific, RPV the Rhopalosiphum padi-specific and PAV the vector nonspecific strain of BYDV.

Table 2. A comparative study of barley yellow dwarf virus strains using ELISA and aphid transmission in west-central Morocco

Groups of Isolates	Number of samples per group	A_{410} using enzyme-labelled globulins shown [a]						Percentage aphid transmission using the indicated aphid species[b]				Similarity to the known BYDV strains[c]
		B	3A11	F	2B12	7F6	6G7	RP	RM	SA	SG	
A	26	2.50	0.85	0.04	0.10	0.12	0.07	60	32	25	15	PAV
B	1	0.76	0.08	0.37	0.04	0.07	0.04	53	0	14	28	PAV+MAV
C	1	1.36	0.20	0.06	0.10	0.41	0.52	43	3	33	27	PAV+RPV
Healthy (control)	-	0.06	0.09	0.10	0.05	0.13	0.08	0	0	0	0	Virus free

[a]Globulins B and 3A11 are, respectively, polyclonal and monoclonal antibodies specific to the PAV strain, F and 2B12 are monoclonal antibodies specific to the MAV strain, 6G7 is a monoclonal antibody specific to the RPV strain, and 7F6 a monoclonal antibody specific to both MAV and RPV strains.

[b]The aphid species used in this comparative test were Rhopalosiphum padi (RP), R. maidis (RM), Sitobion avenae (SA), and Schizaphis graminum (SG). The acquisition access period was 2 days and inoculation access was 5 days using 5 aphids per seedling of Avena sativa var. Clintland'64.

[c]MAV is S. avenae-specific, RPV is the R.padi-specific, and PAV is the vector nonspecific strain of BYDV.

Table 3. Occurrence of barley yellow dwarf virus strains, singly or in combination, as demonstrated by ELISA of field-collected material from west-central Morocco

Virus Isolates[a]	Number of samples belonging to the plant species shown and infected by the virus isolates indicated				
	Wheats	Barley	Corn + sorghum	Grasses[b]	Totals
PAV	140	73	24	25	262
MAV	53	32	6	14	105
RPV	7	7	0	0	14
PAV + MAV	44	32	23	27	126
PAV + RPV	6	1	2	7	16
MAV + RPV	7	2	0	0	9
PAV + MAV + RPV	15	4	3	13	35

[a]Isolate PAV is the vector nonspecific strain, MAV is the S. avenae specific strain, and RPV is the R. padi-specific strain of BYDV.

[b]The grass species included in this study are shown in Table 8.

Table 4. Comparison of ELISA detection of the Moroccan BYDV isolates using antisera to the American and European isolates of the virus

Origin of the anitserum[a]					
Europe		North America			
Desig-nation	Virus detection	Desig-nation	Virus detection	Relative frequency[b]	Cumulated frequency
B	positive	3A11	positive	16.1	
B	positive	3A11	negative	30.5	
B	negative	3A11	positive	7.5	
B	negative	3A11	negative	45.9	
					100.0
F	positive	2B12	positive	6.2	
F	positive	2B12	negative	7.3	
F	negative	2B12	positive	17.2	
F	negative	2B12	negative	69.3	
					100.0

[a]Antibodies B and 3A11 are known to be specific to the non-specific, PAV, strain of BYDV; F and 2B12 are MAV-specific.

[b]The frequencies were calculated on a basis of 1507 entries.

Table 5. Incidence of BYDV in west-central Morocco for 1986-1988

BYD incidence class	Percent fields per class [a]		
	April 86	April 87	March 88
Traces to 10%	36	39	59
11 to 20%	29	15	30
21 to 30%	0	8	8
Over 30%	36	15	2
Large patches [b]	0	25	1

[a]Percent calculated on the basis of 14, 75, and 90 fields for April 86, April 87, and March 88, respectively.

[b]Large patches represent expanded areas of severely damaged plants throughout the fields.

Table 6. Severity patterns of barley yellow dwarf disease of small grains over two growing seasons in west-central Morocco

Growing season	disease severity score[a]	Percent of fields receiving the severity score shown		
		Durum wheat	Bread wheat	Barley
April 1987	0	3	8	0
	1	41	54	26
	2	16	12	22
	3	22	8	17
	4	11	17	22
	5	5	0	4
	6	0	0	4
	7	0	0	4
	8	0	0	0
	9	3	0	0
	Total fields	37	24	23
March 1988	0	14	33	28
	1	7	4	9
	2	59	62	42
	3	9	0	7
	4	2	0	2
	5	2	0	5
	6	5	0	4
	7	0	0	0
	8	0	0	2
	9	0	0	0
	Total fields	42	24	54

[a]The disease severity scores used are those of Schaller and Qualset (28).

Table 7. Occurrence of barley yellow dwarf virus strains and aphid species on oat bait plants at four different locations in west-central Morocco.

Harvest date of bait plants	Aphid species encountered on the bait plants[a]							Virus isolate identified by using ELISA on the plants[b]		
	RP	RM	SA	SG	MD	DN	UN	PAV	MAV	RPV
Dec.86	+	-	-	-	-	-	-	+	+	-
Jan.87	+	-	-	+	-	-	-	+	-	-
Feb.87	+	-	-	-	-	-	-	+	-	-
Mar.87	+	-	+	+	-	+	-	+	-	-
Apr.87	+	-	-	+	-	-	-	+	+	-
May 87	-	-	-	+	-	-	-	+	+	-
Jun.87	+	-	-	-	-	-	-	+	+	+
Jul.87	-	-	-	-	-	-	-	+	+	-
Aug.87	-	-	-	-	-	-	-	+	+	-
Sep.87	+	-	-	-	-	-	-	+	+	-
Oct.87	+	-	-	-	-	-	-	+	+	-
Nov.87	+	-	-	-	-	-	-	+	+	+
Dec.87	+	-	-	-	-	-	-	+	+	+
Jan.88	+	-	-	-	-	-	-	+	+	+
Feb.88	+	+	-	-	-	-	-	+	+	+
Mar.88	+	-	+	-	-	-	-	+	+	+
Apr.88	+	+	+	-	+	-	-	+	+	+
May 88	+	-	-	-	-	-	-	+	+	+
Jun.88	+	-	-	-	-	-	+	+	+	+

[a]The symbols used designate RP = Rhopalosiphum padi, RM = R. maidis, SA = Sitobion avenae, SG = Schizaphis graminum, MD = Metopolophium dirhodum, DN = Diuraphis noxia, and UN = Unknown species. Their presence on the trap plants is designated by the sign +, their absence by - .

[b]The ELISA used in this study was the double antibody sandwich using immunoreagents specific to the vector nonspecific PAV, the R. padi-specific RPV, and the S. avenae-specific MAV strains of BYDV. The presence of any one strain in the bait plants is indicated by the + sign and its absence by the - sign.

Table 8. Grass hosts of barley yellow dwarf virus collected from fields in west-central Morocco during the 1986 to 1988 growing seasons

Grass species collected	Date of collection (month/year)	ELISA reactions of antisera prepared against BYDV strains[a]		
		PAV	MAV	RPV
Aegilops ovata L.	4/87	+[b]	+	+
Arundo donax L.	10/86; 4/87; 2/88; 8/88	+	+	-
Bromus rigidus Roth.	4/87	+	-	-
Cenchrus longispinus (Hack.) Fer.	3/88	+	+	+
Cynodon dactylon (L.) Pers.	6,7 & 10/86; 12/87; 2/88	+	+	+
Digitaria sanguinalis Scop.	3/88	+	+	+
Echinochloa cruss-galli (L.) Beauv.	3/88	+	-	+
Hordeum murinum L.	3/88	+	-	+
Oryzopsis miliacea (L.) Benth and Hook	12/87; 8/88	+	-	+
Paspalum dilatatum Poir.	3/88	+	-	+
Pennisetum villosum R. Br.	6/88	+	-	+
Phalaris brachystachys Link.	3/88	+	-	+
Phalaris paradoxa L.	3/88	+	-	+
Phragmites communis Trint.	12/87; 8/88	+	+	-
Sorghum halepense (L.) Pers.	12/87; 1/88	-	+	-
Unknown species	11/86	+	+	-

[a]The double antibody sandwich ELISA in this study used antibodies prepared against the Rhopalosiphum padi-specific (RPV), the Sitobion avenae-specific (MAV), and the nonspecific (PAV) strains of BYDV.

[b]The symbols, + and - designate positive and negative reactions, respectively.

Table 9. Reactions of some pasture grasses to inoculation by barley yellow dwarf virus

Grass species	Expression of BYDV symptoms by the inoculated plants[a]	Plant samples testing ELISA positive per total number of plants tested[b]
Agropyron cristatum (L.) Gaertn.	Yes	3/6
Agropyron dasystachyum (Hook.) Scribn.	No	1/2
Agropyron desertorum (Fisch.) Schult.	No	1/4
Agropyron elongatum (Host) Beauv.	Yes	3/6
Agropyron smithii Rydb.	Yes	2/3
Agropyron trichophorum (Link.) Richt.	Yes	1/6
Bouteloua curtipendula (Michx.) Torr.	No	0/2
Bromus inermis Leyss	No	4/4
Dactylis glomerata L.	Yes	3/4
Elymus junceus Fsch.	Yes	6/6
Eragrostis intermedia Hitchc.	No	3/5
Eragrostis lehmaniana Nees.	No	0/4
Oryzopsis sp.	Yes	1/1
Phalaris tuberosa (Hack.) Hitchc.	Yes	4/7
Sporobolus airoides (Torr.) Torr.	Yes	4/6
Stenotaphrum secundatum (Walt.) Kuntze	No	1/1
Stipa retorta Cav.	Yes	-

[a]Plants were inoculated with the nonspecific (PAV) strain of BYDV vectored by Rhopalosiphum padi at 5 aphids per plant.

[b]The double antibody sandwich ELISA used in this study used the PAV-specific immunoreagents.

Table 10. Comparison of the reactions of some grasses as possible hosts of the Moroccan BYDV isolates with the results of the previous homologous studies

Grass species tested	Host range comparison for BYDV with previous results[a]		
	immune	Symptomless carriers	Susceptible
Local grass species[b]			
Aegilops ovata			MO
Arundo donax			MO
Bromus rigidus			MO CA WA
Cenchrus longispinus			MO
Cynodon dactylon	AU	CA	MO
Digitaria sanguinalis	CA		MO AU
Echinochloa cruss-galli			MO AU JA
Hordeum murinum			MO GR
Oryzopsis miliacea	AU		MO
Paspalum dilatatum	AU		MO
Pennisetum villosum			MO
Phalaris brachystachys			MO CA
Phalaris paradoxa			MO
Phragmites communis			MO
Sorghum halepense			MO MI
Stenotaphrum secundatus	AU	MO	
Stipa retorta			MO
Grass species introduced[c]			
Agropyron cristatum	CA NY WA		MO KS NY
Agropyron dasystachyum		MO	KS
Agropyron desertorum		MO	
Agropyron elongatum	KS		MO
Agropyron smithii	KS		MO
Agropyron trichophorum	WA	WA	MO
Bouteloua curtipendula	MO	CA	
Bromus inermis	IN WA NY	NY MO	CA
Dactylis glomerata	IN NY	GR WA CA E	MO AU GR WA
Elymus junceus			MO
Eragrostis intermedia		MO	
Eragrostis lehmaniana	MO		
Oryzopsis sp.			MO
Phalaris tuberosa	WA	CA	MO
Sporobolus airoides			MO

[a]Results are fom Australia = AU (12), Japan = JA (14), Greece = GR (22), Kansas = KS (29), Indiana = IN (9), California = CA, Washington = WA, Mississipi = MI, New York = NY, England = E (3), Morocco = MO.

[b]The local grasses were collected outdoors and tested serologically for virus presence.

[c]The grass species introduced were artificially inoculated with the BYDV-PAV strain in the greenhouse.

Figure 1. Map of west central Morocco showing the sites where oat trap plants for BYDV were installed. 1 = Jemaa shaim; 2 = Sidi El Aidi; 3 = Khouribga; 4 = Beni Mellal from West to East

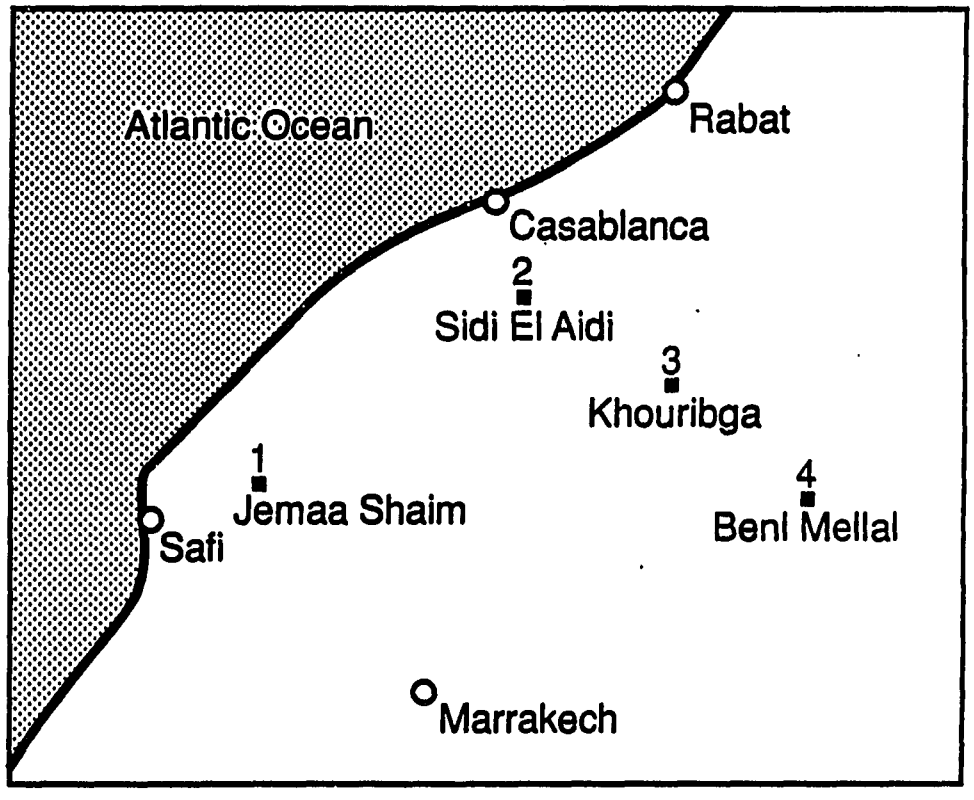
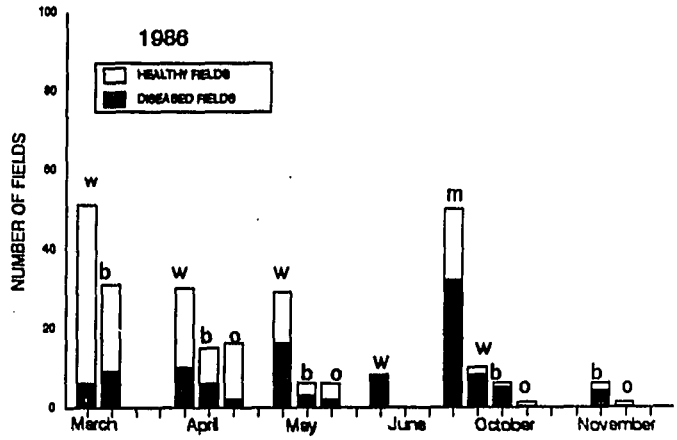
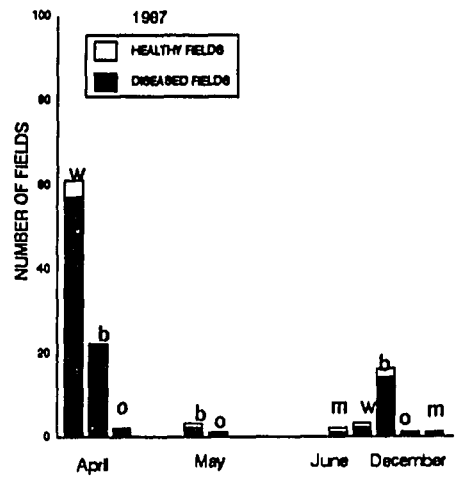
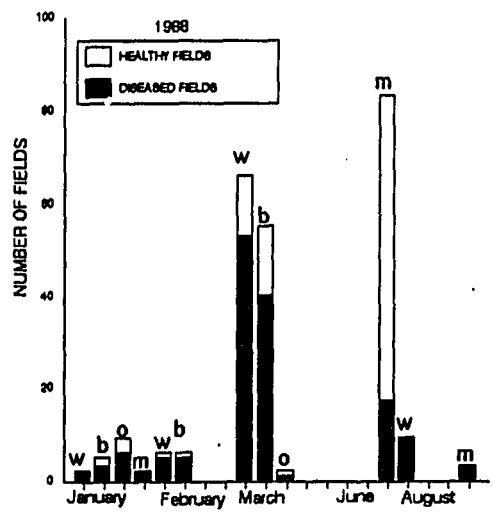

Atlantic Ocean
Rabat
Casablanca
2
Sidi El Aidi
3
Khouribga
1
Jemaa Shaim
4
Beni Mellal
Safi
Marrakech

Figure 2. Incidence of barley yellow dwarf infected fields of wheat (w), barley (b), oats (o), and maize (m) in west central Morocco


1986
HEALTHY FIELDS
DISEASED FIELDS
NUMBER OF FIELDS
March
April
May
June
October
November


1987
HEALTHY FIELDS
DISEASED FIELDS
NUMBER OF FIELDS
April
May
June
December


1988
HEALTHY FIELDS
DISEASED FIELDS
NUMBER OF FIELDS
January
February
March
June
August

SECTION II: APHID SPECIES IN RELATION TO THE TRANSMISSION OF BARLEY YELLOW DWARF VIRUS ISOLATES IN WEST-CENTRAL MOROCCO

Aphid species in relation to the transmission of barley yellow dwarf virus isolates in west-central Morocco

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ABSTRACT

Seven aphid species, collected in west central Morocco, were evaluated for ability to transmit barley yellow dwarf virus (BYDV). Aphids either were collected from grasses showing symptoms of BYDV infection or were allowed acquisition access to plants infected with strain PAV. Immunoreagents to specifically detect the PAV, MAV, and RPV strains of BYDV showed that only Melanaphis donacis was not a vector. A total of 10 grass aphid species in Morocco are now known to act as vectors of BYDV. Three new vectors of BYDV are identified.

INTRODUCTION

Barley yellow dwarf virus (BYDV) is obligately vector-transmitted. All efforts to transmit this virus by other means have been unsuccessful (2). With the exception of a single report on the larvae of the fruit fly, Oscinella frit (L.) from Northern Ireland (9), aphids, which transmit the virus in a persistent manner, are the only natural vectors of the virus.

Historically, five different virus strains have been identified on the basis of vector specificity (15). These are designated as: MAV, Macrosiphum (Sitobion) avenae (Fab.)-specific; RMV, Rhopalosiphum maidis (F.)-specific; RPV, R. padi (L.)-specific; SGV Schizaphis graminum (Rond.)-specific; and PAV, a nonspecific strain of the virus transmitted primarily by R. padi and S. avenae and only occasionally by the other species. Early studies on aphid vectors of BYDV, as summarized by Bruehl (2), recognized only 8 of the 3742 identified aphid species as definitive vectors of the virus. More recently, the number of aphid species recognized as vectors is 17 (1) or 18 (8). In North America, the four species already mentioned and three others, including R. insertum (Walk.), R. rufiabdominalis (Saski), and Metopolophium dirhodum (Walk.), are important vectors (8, 12). In Europe, A'Brook and Dewar (1) and Plumb et al. (13) reported that some 11 aphid species, including Anoecia corni (Fab.), Ceruraphis eriophori (Walk.), Metopolophium albidum (H.R.L), M. dirhodum (Walk.), M. festucae (The.), M. frisicum (H.R.L), R. insertum (Walk.), R. maidis (F.), R. padi (L.), S. avenae (Fab.), and S. fragariae, were important vectors of BYDV in the United Kingdom. R. padi, S. avenae, and M. dirhodum are important vectors in France (7), Spain (14), and

Switzerland (3). The last three species, in addition to S. fragariae, R. maidis, and S. graminum, are important vectors in Italy (11). In North Africa and the Middle East, R. maidis, R. padi, S. avenae, S. graminum, and Sipha (Rungsia) maydis (Pass.) have been reported to transmit BYDV (5, 10). In the southern hemisphere of Africa, R. padi, R. rufiabdominalis, R. maidis, M. dirhodum, S. graminum, S. avenae, Myzus persicae, and Diuraphis noxia (Mordw.) were reported as vectors (19, 20, 21). More recently in Morocco, Sekkat (17) identified a total of 15 aphid species on grasses, including cereals. The status of some of these aphid species as vectors of BYDV is still unknown. The objective of this study was to document the occurrence of previously unreported BYDV vectors in west-central Morocco.

MATERIALS AND METHODS

Aphid transmission

The propagation and transmission of BYDV by the four aphid vectors R. paid, R. maidis, Sitobion avenae, and Schizaphis graminum has been described (5). Seven other aphid species (Table 1) were collected in west-central Morocco to test their ability as virus vectors. Aphids were identified by A. Sekkat (National College of Agriculture, Meknes, Morocco) and tested for transmission of BYDV by using two methods. In the first instance, Anoecia corni (Fab.), Forda marginata (Koch), and Sipha maydis (Pass.) were collected from grass hosts showing symptoms of BYDV and transferred directly to healthy 'Clintland '64' oat (Avena sativa L.) seedlings used as an indicator host. Aphids (5 aphids/seedling) were maintained on the indicator host for at least 5 days, after which plants were sprayed with an insecticide (Methomyl, 25% a.i. at 1.5 g/l of water). Plants were observed for symptom development for at least 4 wk after inoculation. Alternatively, all aphid species, except F. marginata, were collected from the grass hosts and given supplemental feeding for 2 days on Clintland '64 oat leaf pieces infected with a strain of BYDV previously identified as PAV-like (Table 2). The aphids were used to inoculate the indicator hosts as described.

ELISA

After symptoms were recorded, leaves were tested by enzyme-linked immunosorbent assay (ELISA) as described elsewhere (6). Antibodies used were the monoclonal antibodies F and MAV 2B12 specific to the MAV strain, MAV 6G7 specific to the RPV strain, and PAV 3A11 and the polyclonal B

antibody specific to the PAV-strain of BYDV. The monoclonal antibody MAV 3B10 was used as a universal capture for the antibodies 2B12, 6G7, and 3A11. These antibodies were previously produced and characterized at Iowa State University (4, and unpublished data). The antibodies F and B antisera were from BIOREBA (Basel, Switzerland) as kits. $Absorbance_{410}$ values greater than that of healthy sap plus 3 standard deviations were considered positive.

RESULTS

Identification of a PAV-like isolate

Data characterizing the BYDV isolate used in supplemental feeding experiments showed that it was nonspecifically transmitted by R. maidis, S. graminum, R. padi and S. avenae. Parallel serological data for this isolate showed positive reactions with antibodies 3 A11 and B, which are known to be PAV-specific. Antisera specific to either MAV or RPV strains did not react positively with this same isolate. Clintland '64' oat seedlings infested with aphids from virus-free cultures did not show symptoms or react with any of the antibodies used (Table 2).

ELISA

Results of ELISA performed on the aphid-inoculated oat material showed that all aphids tested transmitted BYDV except M. donacis (Table 3). Monoclonal antibody 2B12, prepared by using the MAV isolate of Rochow (15), detected infection by BYDV more frequently than its European F monoclonal antibody counterpart. The converse was true for the European B polyclonal antibody compared with the monoclonal antibody 3A11, which was prepared by using the PAV isolate from Indiana. In general, antibody F detected virus from plants inoculated by five aphid species, 2B12 from those inoculated by six aphid species, 6G7 from plants inoculated by four aphid species, 3A11 from those inoculated by three aphid species, and B from those inoculated by six aphid species. Inoculation by six of the seven aphid species caused plants to test positive with at least two of the panel of five antibodies used.

DISCUSSION

Data obtained for the Moroccan PAV-like isolate of BYDV (Table 2) established the identity of this isolate by aphid transmission and ELISA. It also confirmed transmission of Moroccan isolates of BYDV by M. avenae, R. maidis, R. padi, and S. graminum (5, 10) and demonstrated correlation between BYDV-PAV strain identification by aphid transmission and ELISA (6). The data demonstrate that use of antisera from various sources was necessary because of differences in specificity of the isolates. This phenomenon may be, partly, related to the origin of the virus isolates used to raise the antibodies and epitopic variation that may occur among the isolates.

Data for each aphid species and virus strain, summarized in Table 4, indicate that all aphids, except M. donacis transmitted more than one isolate of BYDV. Apparently, the MAV and PAV strains were transmitted by all six aphid species, but RPV was not transmitted by D. noxia or M. dirhodum (Walk.).

The present status of transmission by aphids of BYDV in Morocco is summarized and compared with previous reports in Table 5. Examination of previous reports (1, 2, 8, 13, 18) revealed that Sipha (Rungsia) maydis, Forda marginata, Melanaphis donacis, and M. pyrarius (Pass.) have been tested only in Morocco. Diuraphis noxia was previously reported by South African workers (19, 20) as an efficient vector of BYDV.

These data demonstrate that at least ten aphid species can transmit BYDV in Morocco. However, the list of proven vectors may still be incomplete because Sekkat (17) reported that at least 15 aphid species

frequent grasses and cereals in Morocco. Several of these have been reported as vectors of BYDV (8, 18).

In our studies, M. donacis did not transmit the PAV strain or other potential strains of BYDV. However, additional studies with other BYDV isolates are necessary before concluding that it is not a vector of BYDV. Similarly, the ability of D. noxia and and M. dirhodum to vector the RPV strain of BYDV requires further examination. This study strengthens the important role that some perennial grasses such as Oryzopsis may play in the epidemiology of BYDV in Morocco. This grass is, not only a host of the virus (6), but also a host for at least two of its vectors (Table 1). In addition, although some of these aphid species, such as Anoecia corni, Forda marginata, and R. padi, complete their life cycles on primary hosts other than grasses or cereals under harsh weather conditions, the mild Mediterranean climate allows them to continue reproducing parthenogenetically (14, 17). This enhances the potential for significant economic loss by epiphytotics of BYDV in most cereal-growing regions of Morocco.

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Table 1. Aphid species used in barley yellow dwarf virus transmission trials

Aphid species used for attempted virus transmission	Plant genus hosting the aphids when collected	Number of transmission trials [a]		Total number of trials
		With supplemental feeding on PAV infected plants	Without any extra feeding on PAV infected plants	
Anoecia corni	Oryzopsis	3	3	6
Diuraphis noxia	Triticum	1	0	1
Forda marginata	Triticum	0	6	6
Melanaphis donacis	Arundo	1	0	1
Melanaphis pyrarius	Oryzopsis	2	0	2
Metopolophium dirhodum	Hordeum	1	0	1
Sipha maydis	Bromus	3	1	4

[a]Supplemental feeding was done by keeping the aphids on BYDV-PAV infected plants for at least 2 days as an acquisition access period before they were transferred onto seedlings of Clintland 64 oats. When no supplemental feeding was used, aphids were directly transferred from plants on which they were found to Clintland 64 seedlings.

Table 2. Biological and serological characterization of the BYDV-PAV isolate used to assess the ability of grass aphid species to transmit BYDV

Isolate type used[a]	Percent transmission of the isolate by the aphids[b]				A_{410} of the reactions between the isolate indicated and the antisera[c]				
					MAV		RPV	PAV	
	RP	RM	SA	SG	F	2B12	6G7	3A11	B
PAV-like	67	10	21	2	0.14	0.07	0.04	0.18	0.54
Controls[d]	0	0	0	0	0.17	0.06	0.06	0.05	0.09

[a]The virus isolate used in these experiments was originally collected from Beni Mellal area.

[b]Transmission experiments consisted of a 2-day acquisition access feeding period on the diseased leaves followed by a 5-day inoculation access period on Clintand '64' oat seedlings using five aphids per seedling of R. padi (RP), R. maidis (RM), S. avenae (SA), and S. graminum (SG).

[c]Antibodies used in the double antibody sandwich ELISA were F and 2B12, MAV-specific, 6G7, RPV-specific, and 3A11 and B, PAV-specific. The antibody concentrations used were 4 μg/ml for 2B12, 0.03 μg/ml for 6G7, and 4 μg/ml for 3A11. Antibody 3B10 was used at 1-4 μg/ml for capture of the previous ones. Antibodies B and F were used at dilutions 1/500 and 1/400 as capture and 1/800 and 1/600 as conjugated antibodies, respectively.

[d]Aphid transmission controls consisted of individuals from virus-free cultures placed on Clintland '64' oat seedlings for inoculation. ELISA controls consisted of healthy sap from Clintland '64' oats used in parallel tests with all the antisera used. ELISA values of the controls represent means of two replications plus three standard deviations for respective antibodies. A_{410} greater than those values are considered positive.

Table 3. Serological evaluation of the ability of grass aphid species to transmit BYDV isolates in west-central Morocco

Aphid species used in the transmission experiment	No of trials[a]	No of trials in which A_{410} was greater than that of the control plus 3 standard deviations for each antibody[b]				
		MAV		RPV	PAV	
		F	2B12	6G7	3A11	B
Anoecia corni	6	2	4	3	4	4
Diuraphis noxia	1	0	1	0	0	1
Forda marginata	6	2	2	3	1	2
Melanaphis donacis	1	0	0	0	0	0
Melanaphis pyrarius	2	1	1	1	0	2
Metopolophium dirhodum	1	1	1	0	0	1
Sipha maydis	4	1	2	1	2	3

[a]Total trials include experiments that used supplemental feeding on PAV-infected plants as well as experiments to transmit BYDV by using aphids collected directly from symptomatic plants in the field. A trial consists of any transmission attempt using the aphid species under study to transmit BYDV from a source to Clintland '64' oat seedlings.

[b]Duplicate samples were tested in all cases. Control A_{410} values including the mean value of duplicate samples plus three standard deviations were 0.11 for F, 0.18 for 2B12 and 6G7, 0.32 for 3A11, and 0.08 for the B antibody. Any A_{410} greater than that of the controls plus three standard deviations was considered positive.

Table 4. Transmission of BYDV isolates by the grass aphid species collected in west-central Morocco as determined by ELISA

Aphid species used in the transmission experiment	No. of trials involving the aphid species[a]	No. of positive aphid transmission trials for the BYDV strains assayed[b]		
		PAV	MAV	RPV
Anoecia corni	6	6	4	3
Diuraphis noxia	1	1	1	0
Forda marginata	6	2	2	3
Melanaphis donacis	1	0	0	0
Melanaphis pyrarius	2	2	1	1
Metopolophium dirhodum	1	1	1	0
Sipha maydis	4	3	3	1

[a]Total trials include experiments that used supplemental feeding on PAV-infected plants as well as experiments using aphids collected directly from symptomatic plants in the field. A trial consists of any transmission attempt using the aphid species under study to transmit BYDV from a source to Clintland '64' oat seedlings.

[b]Results presented are based on ELISA data collected from seedlings of Clintand '64' oats inoculated by the respective aphid species. The antisera used were as described in Table 3.

Table 5. Aphids reported as vectors of barley yellow dwarf virus in west-central Morocco as compared with previous reports

Aphid species identified as vectors of BYDV worldwide	Aphid vectors occurring in Morocco	Serotype(s) of BYDV transmitted in Morocco
Anoecia corni (Fab.)[a]	+	PAV, MAV, RPV
Aphis glycines[b]	-	
Aphis gossypii (Glov.)[b]	+	Not Tested
Aulacorthum circumflexum (Buck.)[ab]	-	
Cavariella salicicola[b]	-	
Ceruraphis eriophori (Walk.)[a]	-	
Diuraphis noxia (Mordow.)[c]	+	PAV, MAV
Forda marginata (Koch)	+	PAV, MAV, RPV
Hyalopterus pruni[b]	+	Not Tested
Longiungus sacchari[b]	-	
Melanaphis donacis	+	None
Melanaphis pyrarius	-	
Metopolophium dirhodum (Walk.)[ab]	+	PAV, MAV
Metopolophium festucae (The.)[a]	-	
Metopolophium frisicum (H. R. L.)[a]	-	
Myzus momonis[b]	-	
Myzus persicae (Sulzer)[ab]	+	Not Tested
Rhopalosiphum insertum (Walk.)[ab]	-	
Rhopalosiphum maidis (Fitch)[ab]	+	PAV
Rhopalosiphum padi(L.)[ab]	+	PAV, RPV
Rhopalosiphum poae (Gill.)[ab]	-	
Rhopalosiphum rufiabdominalis (Sasaki)[ab]	-	
Schizaphis graminum (Rondani)[ab]	+	PAV
Sipha elegans (del Guercio)[ab]	-	
Sipha kurdjimovi[b]	-	
Sipha maydis (Pass.)	+	PAV, MAV, RPV
Sitobion avenae (Fab.)[ab]	+	PAV, MAV
Sitobion fragiriae (Walk.)[a]	+	Not Tested
Sitobion miscanthi (Tak.)[a]	-	

[a]Aphid species reported as vectors of BYDV by Jedlinski (8).

[b]Aphid species reported as vectors of BYDV by Sylvester (18).

[c]Aphid species reported as a vector of BYDV by Von Weckmar (19).

SECTION III: PURIFICATION AND SEROLOGY OF A MOROCCAN ISOLATE OF BARLEY YELLOW DWARF VIRUS

Purification and serology of a Moroccan isolate of barley yellow dwarf virus

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ABSTRACT

A Moroccan PAV-like isolate of barley yellow dwarf virus was purified in Morocco from symptomatic oat plants (Avena sativa L. 'Clintland '64') previously inoculated with viruliferous Rhopalosiphum padi L. The technique included clarification with chloroform, precipitation with polyethylene glycol, and centrifugation through sucrose density gradient columns. The purified virus preparation had an A_{260}/A_{280} of 1.62 and an ultraviolet absorption spectrum characteristic of nucleoproteins. Virus yield averaged 1.45 mg/kg fresh weight of tissue. The virus preparation was used to raise antibodies in rabbits. Antisera had a homologous titer as high as 1/3000 in indirect enzyme-linked immunosorbent assay.

INTRODUCTION

Barley yellow dwarf virus (BYDV), the type member of the luteovirus group, encompasses several strains of isometric phloem-limited, aphid-borne and nonsap-transmissible viruses (12, 14). The first attempts to purify BYDV resulted in very low yields from infected plant tissue (15, 16). Recently, improved purification techniques have increased virus yield substantially (3, 7, 11, 13). The use of macerating enzymes to improve virus yield was described for some luteoviruses (19). Additionally, several variations of the enzyme-linked immunosorbent assay (ELISA) (2, 4, 8, 9) have provided a quantitative serological method for monitoring yields of BYDV. In most cases, ELISA correlates with vector specificity for differentiating among BYDV strains (18), allowing its use as a routine technique for virus identification and assay.

In Morocco, BYDV was identified using aphid transmission. It has since been demonstrated to be important in all the growing areas in Morocco (5, 6, 10). The objective of the present study was to purify a Moroccan isolate of BYDV in Morocco in order to raise specific antiserum for diagnostic purposes.

MATERIALS AND METHODS

Virus origin and purification

The virus isolate used in this study originated from Beni Mellal and reacted as a PAV-like isolate (6). Virus was purified from symptomatic oat plants (Avena sativa L. 'Clintland '64') which had been inoculated at the 2-leaf stage with viruliferous Rhopalosiphum padi L. Oats were grown in a steam-sterilized soil mix. Plants were grown in growth chambers at 15-20 C with a 16-hr photoperiod. In general, the purification technique used was as described by Hammond et al. (7).

Inoculated plants were harvested when symptoms developed after inoculation (3 wk) and stored at -80 C until used. Frozen plant material was ground in 0.5 M sodium phosphate buffer, pH 6.0, (1:3; w/v) containing 0.5 % sodium sulfite 0.01 M diethyldithiocarbamic acid (DIECA). The product was filtered through cheesecloth, a 2:1 mixture of chloroform:n-amylalcohol was added dropwise to one fifth volume, and the mixture was blended for 30 sec. at low speed. After incubation for 60 min at 4 C, the mixture was centrifuged at 7,000 rpm for 10 min in a Beckman JA14 rotor. Sodium chloride and polyethylene glycol (mol. wt = 6,000 M.W.) were added to the supernatant to 0.25 M and 10 %, respectively, with stirring for 90 min at 4 C. Virus was collected by centrifugation at 7,000 rpm for 20 min as above and the pellets were resuspended at 4 C overnight in 10 ml of 0.1 M sodium phosphate, pH 7.0 containing 0.01% NaN_3. After centrifugation at 10,000 rpm for 30 min in the Beckman JA20 rotor, preparations were layered on a 7-ml cushion of 30% sucrose in 0.1 M sodium phosphate, pH 7.0, in Beckman 30 rotor tubes. After centrifugation for 4

hr at 27,000 rpm, pellets were resuspended in 1 ml each of 0.1 M sodium phosphate buffer, pH 7.0, at 4 C. The preparation was centrifuged at 10,000 rpm for 1 min (Beckman JA20 rotor), and fractions of 0.5 to 1 ml each were layered on a 7.5% to 30% (1) linear sucrose density gradient prepared in 0.1 M sodium phosphate, pH 7.0. The preparation was centrifuged for 120 min in a SW41 rotor at 30,000 rpm and fractionated with a density gradient fractionator (ISCO Model 640) coupled to an absorbance/fluorescence detector (ISCO Model UA-5, ISCO, Inc., Lincoln, NE). Recovered virus was diluted with four volumes of 0.1 M sodium phosphate, pH 7.0, and concentrated by centrifugation at 27,000 rpm for 4 hr in a Beckman 30 rotor. Virus was resuspended in 0.1 M sodium phosphate, pH 7.0, and centrifuged at 10,000 rpm for 30 min in the Beckman JA20 rotor. Virus concentration was determined spectrophotometrically with an assumed extinction coefficient of $E_{260}^{0.1\%} = 6.0$ (13). The virus preparations were stored at -25 C in the presence of 0.01% NaN_3 (17).

Antiserum preparation

One hundred μg of purified virus antigen mixed with Freund's complete adjuvant at a ratio of 1:1 (1/c) was injected intramuscularly into two rabbits at three weekly intervals. Animals were exsanguinated weekly by cardiac puncture 2 weeks following the last injection. Antiserum titer was determined by indirect ELISA.

Indirect enzyme-linked immunosorbent assay (I-ELISA)

I-ELISA was similar to procedures described elsewhere (4, 9). Wells of Immulon 1 or 2 (Dynatech Laboratories, Chantilly, VA) microliter plates were coated with virus antigen (5 μg/ml, 100 μl/well) in 0.05 M

carbonate-bicarbonate, pH 9.6, and incubated overnight at 4 C. After plates were washed three times, 4 min each, with wash buffer (0.01 M phosphate buffered saline [PBS], pH 7.4, containing 0.85% NaCl, 0.05% Tween 20 and 0.02% KCl), unbound sites in the wells were blocked for 2 hr at room temperature with 400 µl per well of PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween 20. The plates were washed as just described, and antiserum (in 0.01 M PBS-Tween 20 containing 2% polyvinylpyrrolidone, MW 40,000, and 0.2% BSA) was added (100 µl/well) in two adjacent wells. After a 4-hr incubation at 37 C, the plates were washed, and alkaline phophatase-conjugated (100 µl/well) goat-anti-rabbit polyclonal IgG (Sigma Chemical Co., St. Louis, MO, A-8025) diluted 1/500 in PBS (100 µl/well) was added. The plates were incubated for 4 hr at 37 C; after the plates were washed, p-nitrophenyl phosphate (1 mg/ml in 10% diethanolamine, pH 9.8) was added (100 µl/well), and plates were incubated for 30-60 min at 37 C. Reaction products were measured at 410 nm with a Minireader II (Dynatech Laboratory, Inc., Chantilly, VA). Healthy plant sap from Clintland '64' oats was used as a control. Additional controls were preimmune sera from the two rabbits.

RESULTS

Virus purification

Virus preparations usually yielded one visible band (peak I) at about 4 cm from the meniscus of the sucrose density gradient column following centrifugation (Fig. 1 A). Occasionally (1 of 3 purification attempts), a small minor peak (peak II, Fig. 1 B) was evident in all the tubes scanned. In further tests, peaks II reacted only with antibodies raised against the nonspecific PAV-like strain of BYDV in a direct ELISA described elsewhere (6). Purified virus preparations had an ultraviolet absorption spectrum characteristic of nucleoproteins with an absorption maximum at 260 nm and a minimum at 240 nm (Fig. 2). The A_{260} /A_{280} of the three virus preparations were 1.70, 1.63, and 1.53, which gave a mean value of 1.62. Virus yields ranged from 1.25 to 1.65 mg/kg, with an average of 1.45 mg/kg fresh weight of oat plant tissue

Antiserum preparation.

Table 1 summarizes the characteristics of the antisera produced by the two rabbits 17 and 27 days following the last injection. Only one rabbit survived the first bleeding, which permitted only one second bleeding. Reaction products, as measured by A_{410}, in the presence of the virus were 3 to 4-fold greater than those obtained in the presence of healthy oat sap. On the basis of the criterion that A_{410} values greater than that of healthy sap plus three standard deviations are considered positive (4), it was concluded that the antisera harvested after 17 days had a 1/1000 titer and that harvested after 27 days had at least a 1/3000 titer, which represented the highest dilutions used in these trials. Preimmune sera from both

rabbits included in the tests as controls gave low reactions with healthy sap (A_{410} = 0.03 and 0.04 O.D.) as well as with pure virus (A_{410} = 0.04 and 0.05 O.D.).

DISCUSSION

A PAV-like isolate of BYDV from Morocco has been successfully purified. The ultraviolet absorption profiles of scanned sucrose density gradient tubes containing preparations of this isolate as well as ratios (A_{260}/A_{280} = 1.53 to 1.70) performed on pure virus during this study were similar to those reported elsewhere for this strain of BYDV. Indeed, absorption ratios of 1.89 and 1.76 were reported for Illinois and Indiana isolates, respectively (3, 7), in the United States, 1.71 for a Japanese isolate (11), and 1.71 to 1.79 for a Canadian isolate of the same strain of BYDV (13). The peak II in Fig. 1B could have originated from denaturation of virions, giving empty shells that migrated less rapidly in the sucrose columns compared with entire virus particles. This is consistent with the fact that preparations of peak II reacted positively in direct ELISA with antisera against a PAV-like isolate of BYDV. Our virus yield, which averaged 1.45 mg/kg fresh weight of Clintland '64' oats, was reasonably high. However, this yield is still low compared with an average of 4.4 mg/kg fresh weight of root tissue obtained by D'Arcy et al. (3). Since ELISA was adapted for plant viruses by Clark and Adams (2), several other forms of this technique, including the indirect ELISA, have been developed. Lommel et al. (9) demonstrated the usefulness of the indirect ELISA for studying carnation viruses. Diaco et al. (4) studied serological relationships among BYDV. They reported that variation in antibody specificity was dependent upon the use of directly labelled or unlabelled antibodies. The present study supports the use of indirect ELISA as a rapid and effective detection technique. Antiserum titers obtained in this

study were very encouraging; thus, an antiserum is now available to the most common Moroccan isolate of BYDV (PAV). Consequently, ELISA will make routine diagnosis of this isolate possible in Morocco. This will augment laborious aphid transmission techniques (18). Efforts are continuing to obtain similar results for the other BYDV strains present in the country.

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Table 1. Titration of antisera prepared against a Moroccan PAV-like isolate of barley yellow dwarf virus by using the indirect enzyme-linked immunosorbent assay (I-ELISA)

Time between last injection and exsanguination	Animal used	Antigen used	A_{410}[a] Reciprocal of antiserum dilution (x 10) 10	20	30	40	50	60	70	80	90	100
17 days	Rabbit I	Pure virus (500ng/well)	0.24	0.19	0.17	0.19	0.19	0.17	0.17	0.17	0.17	0.16
		Healthy sap	0.05	0.04	0.06	0.05	0.05	0.06	0.04	0.04	0.04	0.04
	Rabbit II	Pure virus (500ng/well)	0.33	0.30	0.25	0.28	0.23	0.28	0.25	0.25	0.20	0.19
		Healthy sap	0.04	0.04	0.04	0.05	0.06	0.04	0.04	0.04	0.04	0.04

			Reciprocal of antiserum dilution (x 10) 50	100	150	200	250	300
27 days	Rabbit II	Pure virus (500ng/well)	0.63	0.42	0.30	0.25	0.20	0.19
		Healthy sap	0.15	0.13	0.14	0.14	0.09	0.07

[a] A_{410} readings are the mean value of two values per dilution.

Figure 1. Ultraviolet absorption profiles of barley yellow dwarf virus preparations purified from cultivar Clintland '64 oats and separated on linear 7.5% - 30% sucrose gradients in 0.1 M sodium phosphate, pH 7.0, centrifuged at 30,000 rpm for 3 hr in a Beckman SW41 rotor. Generally a single peak was obtained (A), but occasionally, a second peak was observed (B).

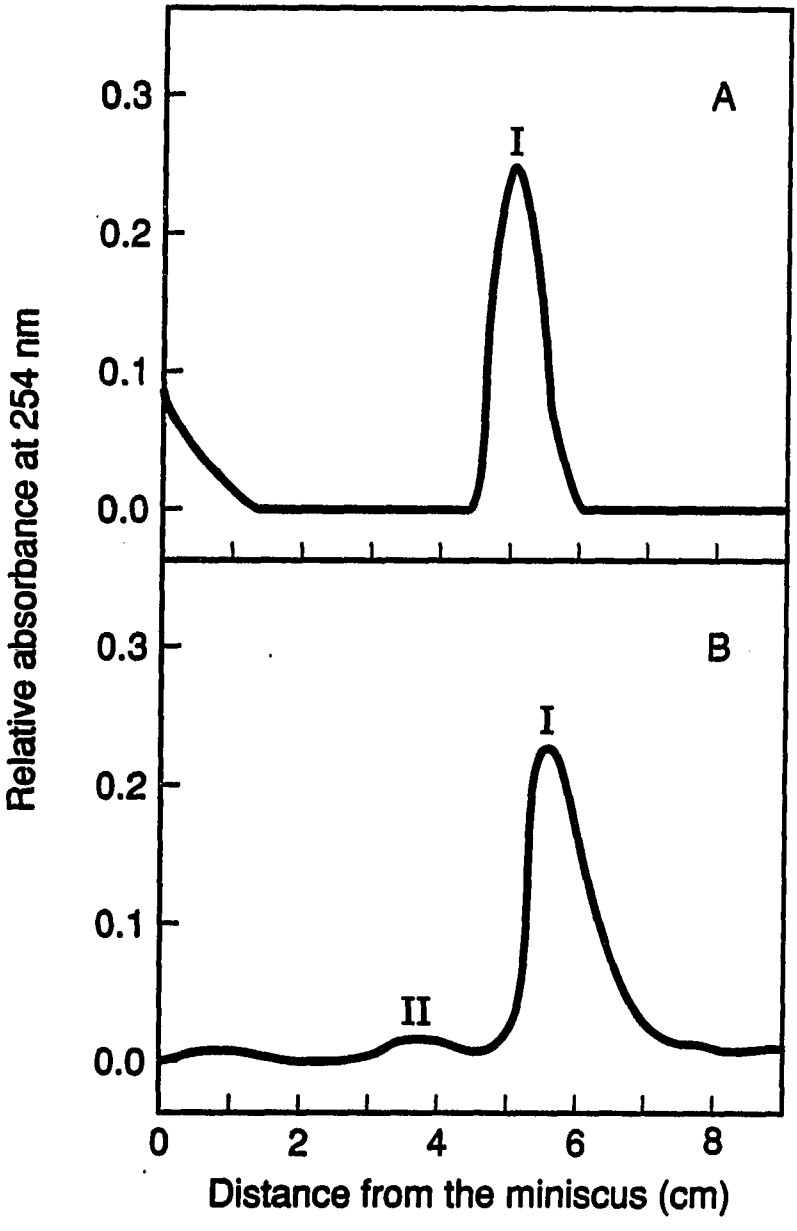
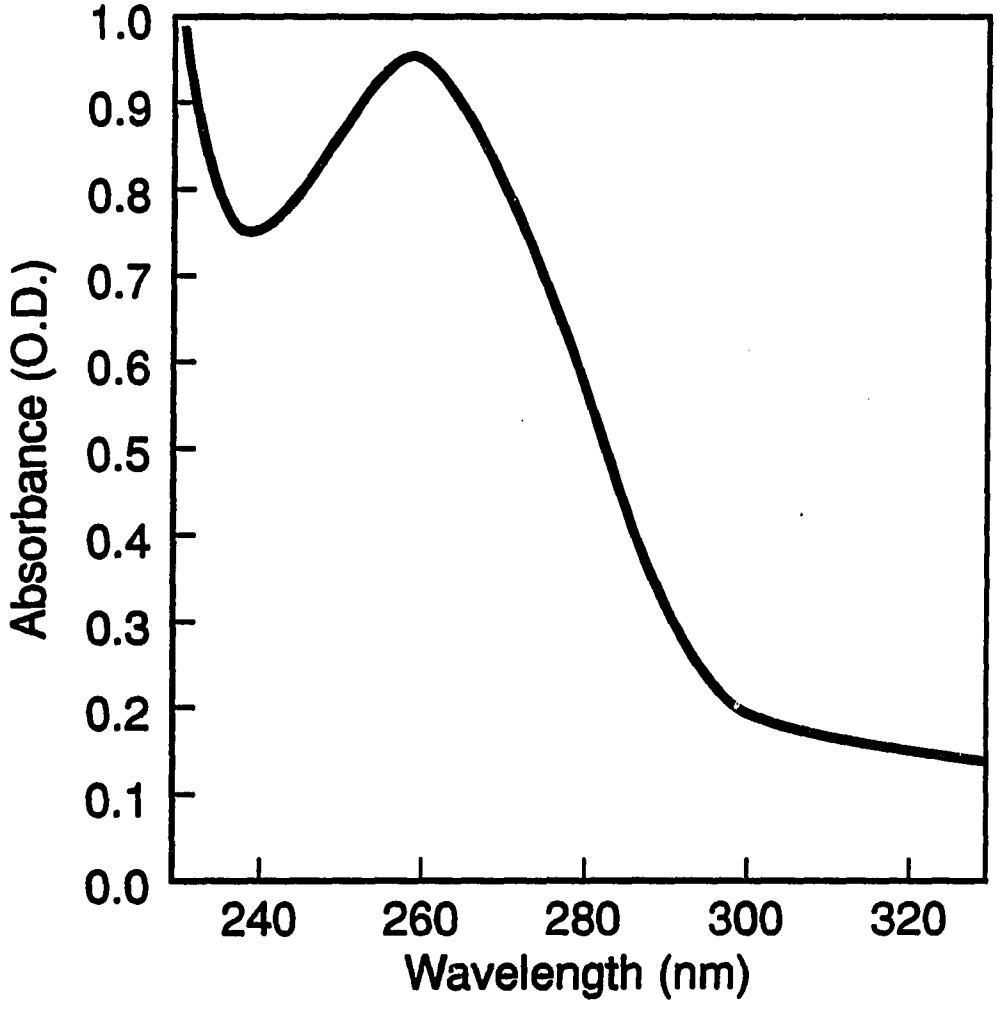

A
B
I
I
II
0.3
0.2
0.1
0.0
0.3
0.2
0.1
0.0
0
2
4
6
8
Relative absorbance at 254 nm
Distance from the miniscus (cm)

Figure 2. Ultraviolet absorption spectrum of a purified preparation of PAV-BYDV obtained from infected Clintland '64 oat tissue


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance (O.D.)
240
260
280
300
320
Wavelength (nm)

SECTION IV: CROP LOSS ASSESSMENT AND GERMPLASM SCREENING FOR RESISTANCE TO BARLEY YELLOW DWARF VIRUS IN WEST-CENTRAL MOROCCO

Crop loss assessment and germplasm screening for resistance to barley yellow dwarf virus in west-central Morocco

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ABSTRACT

Yield losses induced by inoculation of the bread wheat cultivars 'Nesma 149' and 'Saada' with BYDV over 2 growing seasons reached high as 39% , 24%, 38%, and 61% for head and thousand kernel weights, and total and grain yields, respectively. Corresponding losses induced by natural infection were 11%, 11%, 14%, and 26%, respectively. Nesma 149 showed more tolerance to natural virus infection than Saada during the epiphytotic of 1986-87 regarding biological and grain yields. Studies to assess tolerance to BYDV demonstrated that 3 of 8 durum wheat, 4 of 19 bread wheat, 3 of 8 oat and none of 17 barley cultivars tested showed appreciable tolerance to the PAV strain of BYDV.

INTRODUCTION

Barley yellow dwarf (BYD) is a cereal disease of worldwide distribution (23). Economically important losses of grain yield as high as 79% have been reported in major cereal producing countries (3, 7, 12, 29). In yield loss trials using near-isogenic barley lines, the resistant line containing the Yd_2 gene outyielded the susceptible line by 38% (17). These are a few of numerous examples that demonstrate yield loss that can result from BYDV infection. More extensive information is available in proceedings of diverse BYDV workshops (e.g., the 1983 and 1987 BYDV-workshops held in Mexico and Italy, respectively).

Several control practices have been developed (3, 7), but varietal resitance has been the preferred practice, particularly in developing countries.

Barley yellow dwarf was recently recognized as an important disease in all cereals grown in Morocco (16). The purpose of this study was to establish the severity of losses due to BYDV that might be expected under Moroccan conditions and to assess potential sources of resistance or tolerance to BYDV in breeding lines currently used in Morocco.

MATERIALS AND METHODS

Crop loss assessment in Morocco

Field trials to estimate loss in bread wheat yields due to BYDV infection were conducted during 1986-87 and 1987-88. The bread wheat cultivars 'Saada' and 'Nesma 149' were planted at three locations, Sidi El Aidi, Jemaa Shaim and Tessaout during late November 1986 and 1987. At each site a split-plot design was used, comprising 3 randomized blocks. Cultivars were the main plots and subplots included the 3 treatments of untreated control, an insecticide-sprayed plot, and a virus-inoculated plot. Each subplot comprised 6 rows wide, 6 m long, with 30 cm between adjacent rows. Seeds were planted at the rate of 100 kg/ha. Interplot and interblock areas were kept free of vegetation by regular plowing. The insecticide treatment used Deltamethrine (2.5% a.i.) applied at 7.5 g/ha every 3 weeks until heading. Virus-infected plots were inoculated at plant growth stage 2 to 3 (Feekes scale, 14) with a PAV-like isolate (11) by chopping virus-infected oats (Avena sativa L. cv. 'Clintland '64') infested with viruliferous Rhopalosiphum padi L. and distributing the small leaf pieces on plots on windless days. The first insecticide treatment was applied to insecticide-treated plots at the same time as inoculation. Postemergence counts of seedlings were carried out at all locations. Homogeneous stands of 24-28 seedlings per meter of row were established.

Data were obtained from the 4 central rows of each subplot, and included the visual assessment of number of diseased plants per linear meter of row at growth stages 9 to 10 on the Feekes scale; the proportion of heads having blasted terminal florets at stage 11; the head weight at

ripening; total biomass of above ground plant parts; grain yield at harvest (June) as well as the thousand kernel weight. Individual and combined analyses (30) of the data were carried out using Statistical Analysis System (SAS, SAS Institute Inc., Cary, NC) procedures.

Germplasm evaluation and screening

The PAV-like isolate and R. padi previously described (11) were used in these experiments. Seeds of 17 barley, 8 oat, 8 durum, and 19 bread wheat cultivars (Tables 1, 2, 3, 4) were planted in sterile soil in the greenhouse at a rate of 10 to 20 seeds in bottomless plastic bags 20 cm in diameter and 60 cm high.

Viruliferous R. padi, reared on Clintland'64 oats infected with the PAV-like isolate, were collected by shaking infested plants over a funnel. Insects were transferred into flasks containing a little carborundum, which were agitated to avoid clump formation of aphids (8). The aphids (5 aphids/seedling) were applied to the seedlings and the plastic bags were closed to form a cage. Following a 5-day inoculation access period, the bags were trimmed and plants were treated with Deltamethrine. Control plants were plants of the same size treated identically except that no aphids were applied to them. Plants were transferred outdoors and arranged in a split-plot design. Varieties, separated by 50 cm, represented the main plots, which were separated by one meter. Virus inoculated and noninoculated treatments were subplots within each main plot. The experiment was replicated three times. Insecticide applications were made at 3 wk intervals. Data measured were the plant size at stage 10 on Feekes scale (14), disease severity index at stage 10 based on a scale of zero (no

symptoms) to 9 (most severe symptoms) (24), and the biological and grain yields per hill at harvest. Data were analyzed by standard analysis of variance procedures (30). The cultivars Elmo and Caldwell were not included in the analysis because of their winter habits as compared with the other bread wheats.

The double antibody sandwich ELISA, as described previously (11) was performed on samples from all the hill plots in order to monitor virus presence in the artificially inoculated as well as noninoculated plants. Immunoreagents used were to the European "B" or PAV strain of BYDV (BIOREBA, Basel, Switzerland).

RESULTS

Crop loss assessment in Morocco

Factorial analysis and F tests (Appendix V) showed that varieties yielded better in 1987-88 than in 1986-87. No significant effect of location on variety performance occurred. Differences between the effects of the three treatments were highly significant (P = 0.01), for all the plant characters assessed, as demonstrated by the contrasts between the nontreated and insecticide-treated plots and the BYDV-inoculated and both of the other treatments. A significant interaction between virus treatments and location for the head filling as well as yield characters was caused by the fact that disease conditions were more severe at the Jemaa Shaim station than at the other two stations. Also, a significant interaction between the virus treatments, the varieties and the years (P = 0.05) showed that both biological and grain yields of Nesma 149 were less affected by naturally occurring virus than those of Saada during 1986-87 (Fig. 1 C, D, Appendices VIII and IX). Differences in treatment effects on the head weight parameter were enhanced in 1987-88.

Virus inoculation induced 2.5 and 1.7 times the number of diseased plants and 6 and 2.8 times the number of heads with blasted terminal spikelets as compared to insecticide treated or untreated plots, respectively. The insecticide spray reduced the number of diseased plants by 1.5 and the number of heads with blased florets by 2 as compared to the untreated plots (Table 5).

Natural infection with BYDV that occurred in the untreated plots decreased the head weight of both varieties by 10 to 13% in both seasons

except for Saada in 1986-87 as compared to the insecticide treatment. Comparative effects of artificial inoculation with BYDV were reductions of 24 to 42 for Nesma 149 and 13 to 37% for Saada depending on the growing season (Fig. 1, A, Appendix VI).

Natural infection with BYDV resulted in significant reductions in 1000 kernel weight as compared to insecticide treated plants (Fig. 1, B, Appendix VII). Both varieties had a 13% reduction in 1000 kernel weight in 1986-87 and only 3 to 7% in 1987-88 growing season. The potential loss for this trait as measured by the difference between insecticide protected and artificially inoculated plots ranged from 17 to 24% for both varieties in both seasons.

Biological yields from artificially inoculated plots were consistently lower than the other treatments with potential losses ranging from 27 to 38% for Nesma 149 and 23 to 24% for Saada when virus inoculated and insecticide treated plots were compared. Reduction induced by natural infections varied from none to 14% for Nesma 149 and from 11 to 4% for Saada during the 1986-87 and 1987-88 growing seasons, respectively (Fig. 1, C, Appendix VIII). Measurement of grain yield (Fig. 1, D, Appendix IX) showed that during 1986-87, performance of Saada was enhanced by insecticide treatment by 26% when Nesma 149 showed some field tolerance with minimum yield loss due to natural infection with virus. In 1987-88 when disease pressure was less, grain yield losses of 15% and 6% were measured for Nesma 149 and Saada, respectively, as calculated from comparison of untreated and insecticide treated plots (Fig. 1, D, Appendix IX). Potential grain yield losses for the 2 cultivars, as shown by

comparison of the insecticide treated and the virus inoculated plot yields, ranged from 48 to 61% for Nesma 149 and from 51 to 49% for Saada during the two seasons.

Germplasm evaluation and screening

For all the plant material tested, factorial analysis and F tests (Appendix X) showed that the effects of variety as well as inoculation treatments were highly significant ($P = 0.01$) for all the plant characters assessed. An exception to this, however, was the lack of variety effect on the biological and grain yields of bread wheats. Also, the severity of the disease, as measured by disease severity index, was variety dependent. Significant interaction between the variety and the inoculation treatments was measured only for the plant height characteristic of the oat varieties (Table 6, Appendix X). The effect of virus inoculation on biological and grain yields was variety dependent for all the 4 cereals (Table 6, Appendix X), except for the grain yield of the bread wheat varieties. Mean comparisons between the effects of the 2 virus inoculation treatments on the different plant characters are reported in Tables 1, 2, 3, 4. These data, representing mean differences for all plant characters between BYDV-inoculated and insecticide-protected plots, are arranged in increasing order of biological yield mean differences because of the high statistical correlation found between biological and grain yields ($r^2 = 0.90$).

On the basis of biological yield, the cultivars Cocorit and ACSAD 65 showed some tolerance to the virus ($P = 0.05$). At $P = 0.01$, the cultivar Marzak could be considered among the tolerant cultivars. These cultivars also showed some tolerance as measured by grain yield ($P = 0.01$) and had

lower disease severity scores. No tolerance was demonstrated, as assessed by plant height, in any of these cultivars except for Cocorit (Table 1).

In contrast to the durum wheats, comparison of biological and grain yield means of the different bread wheats (Table 2) suggested that the varieties, ACSAD 59 and Jouda and the lines BT.J.15 and BT.J.16, exhibited some tolerance to BYDV ($P = 0.01$). The same varieties and lines also scored low in disease severity (2.3 - 4.0) but plant height was adversely affected by the virus. Examination of barley cultivars tested revealed that no variety gave a mean difference value lower than those accepted at $P = 0.05$ or even at $P = 0.01$ regarding biological yield or plant height and disease severity scores were high. However, based on grain yield reduction, due to inoculation with the virus, the cultivars Harmal, ACSAD 60, and Mona x Maris Dingo showed some tolerance (Table 3).

Mean differences for the different characteristics measured for the oat varieties (Table 4) revealed that the first six of eight varieties showed some tolerance to the virus as expressed by the biological yield. Only three or four of these showed promising tolerance when grain yield was considered. For plant height, only Albion and CI 5068 demonstrated tolerance. All the varieties except Clintland '64, a susceptible control, and Romani 153, had a disease index of 4 or less when inoculated with BYDV.

ELISA of duplicate samples from every hill plot in this study showed that most uninoculated plots remained free of virus infection while the inoculated plots tested positive (data not shown). Plants in uninoculated plots where virus was detected (mostly oats and wheat) remained symptomless.

DISCUSSION

Results of this study have provided an assessment of current and potential yield loss induced by infection of the principal cereals with BYDV, and the evaluation of genetic material from diverse locations, for resistance to BYDV in Morocco. The data obtained for Nesma 149 and Saada are of particular relevance because the former is an adapted Moroccan variety widely grown in the area and the latter is highly resistant to Hessian fly and is considered an important future variety until constraints imposed by the Hessian fly in the area are overcome (15).

Plants were inoculated at development stages 2-3 on Feekes scale (14) based upon previous reports (9, 10, 29). When plants were inoculated both varieties were highly susceptible regardless of the environmental conditions of the experiments. Losses induced by artificial inoculation for all the yield components assessed, especially those of 48 to 61% and 49 to 51% recorded for grain yields of Nesma 149 and Saada, respectively, were similar to losses reported earlier (2, 4, 7, 9, 10, 12, 17, 29, 31). Although we demonstrate that the cultivars 'Nesma 149' and 'Saada' are very susceptible to the PAV isolate of BYDV used, it is dangerous to generalize these data to other BYDV strains (2).

The effect of naturally occurring virus on the yield components assessed was significantly influenced by variety and growth conditions during both seasons. Nesma 149 showed some field tolerance to the virus, as expressed by its biological and grain yields, under drought (Appendix IV) and heavy disease pressure (11) during the 1986-87 growing season (Fig. 1, C, D, Appendices VIII and IX). The data cannot be explained by a

differential effect of Hessian fly on the two cultivars. The fly infestation was similar in both years of this study (15, and unpublished observations). Comeau (9) suggested that "wheat has a temporary rise of resistance at the end of tillering, followed by a period of higher sensitivity during stem elongation, and a second increase in resistance about flowering time". If plants of Nesma 149 were inoculated by naturally-occurring BYDV at a resistant stage of development in 1986-87, the data support the observations of Comeau. This hypothesis is further supported by previous findings of no effect of natural virus infection on yield and the other parameters measured for Nesma 149 during the 1981-82 growing season (10). Nesma 149 is an old variety well adapted to Moroccan growing conditions (Table 2) as compared to Saada, which is a variety that originated from South Dakota and has been newly adapted for Morocco because of its Hessian fly resistance.

Also, the BYD tends to be more severe at the Jemaa Shaim station. Severity of the disease at the Jemaa Shaim station may be attributed to the role of maize as an oversummering host and reservoir for the virus and its vector species as well as cereal and legume mixtures used for forage and cereal volunteer plants which are numerous in this area. The role of maize in the epidemiology of BYDV in Morocco has been demonstrated previously (11). Jemaa Shaim belongs to the Abda region of Morocco and is well-known for large hectarage sown to maize. At present, this crop is enhanced by artificial irrigation practiced in this region and the neighboring Doukkala region.

Although numerous reports concerning the adverse effects of artificial inoculation on yield components have been cited previously (2, 4, 9, 10, 12, 17, 29, 31), this is the first time that yield losses induced by natural virus infections in North Africa, in general, and in Morocco in particular, have been documented. Therefore, these results document the importance of BYD disease in the cereal cropping region of this part of the world.

Numerous control measures for BYD have been described (3, 7). Results from this study suggest considerable benefit from control of aphid vectors. Additional information is necessary, however, before recommendations can be made. Crop management techniques lack flexitbility in Morocco because of the limited and irregular rainfall (<400 mm); at the same time weather is conducive to aphid survival and movement (1, 4, 27). Improvement of harvesting techniques of cereals, search for other forage options to replace mixtures of legume and cereals, complete control of grass weeds and destruction of maize residues would reduce the amount of inoculum which survives from one season to the next. Although these measures may assist control of BYD in west-central Morocco, development of disease resistant cereals will provide the most effective means of control. Therefore, the present data represent the first evaluation of plant material for resistance to the virus in Morocco. Plants were inoculated with the PAV isolate because, according to Rochow (22), isolates of this strain are the most severe ones among the isolates identified. Therefore, varieties selected under these conditions may be useful although mixed

infections, as documented by Baltenberger et al. (2) for PAV and RPV, may break host tolerance.

Among all the parameters measured, biological yield was most highly correlated with grain yield ($r^2 = 0.90$). This was followed by plant size or height ($r^2 = 0.74$) and the disease severity index ($r^2 = 0.64$). Although these correlation coefficients were highly significant ($P = 0.01$), the relatively low correlation coefficient for disease severity index agreed with reports of Skaria et al. (28) demonstrating poor agreement between visual assessment of resistance and reduction in grain yield. This was particularly evident in our studies where a wide disparity occurred between disease severity index and yield reduction for the bread wheats. Although hill plots provide only a preliminary assessment of varietal performance, the high correlation between biological and grain yield made biological yield a primary criterion for assessment of tolerance to virus infection.

The cultivar, Cocorit, is the only durum wheat that showed no significant difference between the virus inoculated and insecticide treated plants for all the parameters assessed. In addition, visible symptoms were mild. If the allowable difference between treatments is increased ($P = 0.01$), the cultivars ACSAD 65 and Marzak demonstrated some tolerance. Similarly, ACSAD 59 was the only bread wheat which demonstrated tolerance for all the traits measured except plant height. However, the lines B.T.J15 and B.T.J16 and the cultivar Jouda may also be useful tolerant material in the future. The cultivar Laudrina, considered virus tolerant in the United States (H. Ohm and J. Foster, Purdue Univ., West Lafayette, IN, personal communication), did not exhibit significant tolerance to the

Moroccan isolate used in this study although it scored less than 3 on the disease index. Significantly, the Hessian fly, Mayetiola destructor Say (15), resistant cultivar, Saada, was highly susceptible to the virus. Therefore, caution should be exercized before this variety is widely grown for resistance to the Hessian fly. The bread wheat cultivars Caldwell (20) and Elmo, resistant to BYDV in the U.S.A. (18) showed only mild symptoms after inoculation (D.I. = 2.33 and 2.00, respectively). Unfortunately, because of their winter growth habit, no other measurement could be obtained. However, these cultivars should be considered for use in the wheat growing areas of the Atlas mountains where tempertures are cold enough to induce vernalization. These cultivars are also resistant to the American biotypes of Hessian fly. The resistance of Elmo to BYDV is, in part, due to Agropyron elongatum (Host) Beauv. (18). In our studies, A. elongatum showed some visible symptoms and tested positive in ELISA (11).

Little tolerance was demonstrated in barley as measured by biological yield, disease severity index, or plant height. However, grain yield showed some potential tolerance as exemplified by Mona x Maris Dingo, Harmel, and ACSAD 60. These results are unsatisfactory, however, because tolerance in barley is controlled by a single incompletely dominant resistance gene named Yd_2 (25) derived from Ethiopian barleys (21, 26). This gene should be incorporated into varieties adapted to Morocco.

The data revealed that Romani 153, a locally-grown oat variety, is as or more susceptible than Clintland '64 for some evaluated traits. Our results for Saia and Otee were consistent with previous reports (5, 6, 13, 19). Our results, regarding the disease severity index based on visual

scoring of the symptoms agreed with those reported earlier for the same varieties (5, 6, 13, 19). The results suggest that the tolerant oat varieties could be substituted for the local susceptible variety, Romani 153.

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Table 1. Mean differences between BYDV-inoculated and control durum wheat varieties for disease severity index, plant height, biological and grain yield (numbers in parentheses represent the performance of the virus inoculated treatments as a percent of the controls)

Variety	Release date[a]	Relative crop importance[b]	Source of the seed[c]	Biological yield (g)	Grain yield (g)	Plant height (cm)[d]	Disease index (0-9)[d]
Cocorit	1975	3	INRA	26(49)	12(34)	13(75)	3
ACSAD 65	1984	0	INRA	37(46)	11(25)	22(64)	4
Marzak	1984	0	INRA	38(46)	20(39)	14(76)	2
Jori	1976	2	INRA	58(19)	24(8)	21(67)	4
Karim	1985	0	INRA	85(18)	33(13)	26(49)	5
Selbera	1930	0	INRA	91(12)	8(0)	24(51)	5
Kyperouda	1956	2	INRA	125(21)	21(17)	19(69)	4
Oued Zenati	1930	1	INRA	148(12)	15(6)	32(48)	6
LSD 5%				38	9	9	-[e]
1%				52	13	13	-

[a]The release was made by the Moroccan Ministry of Agriculture and the Agrarian Reforme on dates indicated.

[b]Hectarage covered by the variety: 0 = 1 to 5%, 1 = 5.1 to 15%, 2 = 15.1 to 30%, and 3 = >30% in Morocco.

[c]The origin was INRA (National Agronomic Research Institute, Cereal Breeding Section, Morocco).

[d]Plant height and disease severity readings were measured at plant growth stage 10 on Feekes scale (20).

[e](-) indicates data not available.

Table 2. Mean differences between BYDV-inoculated and control bread wheat varieties for disease severity index, plant height, biological and grain yield (numbers in parentheses represent the performance of the virus inoculated treatments as a percent of the controls)

Variety	Release date[a]	Relative crop importance[b]	Source of the seed[c]	Biological yield (g)	Grain yield (g)	Plant height (cm)[d]	Disease index (0-9)[d]
ACSAD 59	1985	0	INRA	27(67)	6(61)	17(72)	2
BT J 15	ny[e]	0	INRA	40(58)	10(42)	16(72)	3
Jouda	1984	0	INRA	48(47)	10(45)	20(72)	3
BT J 16	ny	0	INRA	52(28)	10(26)	23(62)	4
Nesma 149	1973	3	INRA	53(32)	13(17)	25(63)	4
Merchouch 8	1985	0	INRA	55(34)	14(31)	19(71)	3
Potam	1975	0	INRA	58(30)	15(23)	14(78)	3
Laudrina	-[f]	0	P.Univ	62(36)	15(15)	27(58)	3
Sais (1615)	1985	0	INRA	63(29)	11(29)	18(73)	3
Siete Cerros	1968	2	INRA	63(32)	16(19)	20(68)	4
BT J 14	ny	0	INRA	68(34)	13(16)	24(56)	5
Merchouch 9	1984	0	INRA	68(25)	12(23)	20(66)	4
Pinyte(2306)	1956	0	INRA	77(28)	11(37)	25(67)	4
Saada	1989	0	INRA	80(9)	15(6)	39(45)	7
BT J 17	ny	0	INRA	84(17)	13(10)	28(50)	5
BT J 13	ny	0	INRA	87(17)	17(7)	20(66)	4
Tegyey (32)	1976	1	INRA	111(10)	21(3)	24(51)	5
Caldwell	1982	0	P.Univ	-	-	-	2
Elmo	1982	0	P.Univ	-	-	-	2
LSD 5%				38	8	10	-
1%				52	11	14	-

[a]The release was made by the Moroccan Ministry of Agriculture and the Agrarian Reforme on dates indicated.

[b]Hectarage covered by the variety: 0 = 1 to 5%, 1 = 5.1 to 15%, 2 = 15.1 to 30%, and 3 = >30% in Morocco.

[c]The origin was Purdue University (H. Ohm and J. Foster), or INRA (National Agronomic Research Institute, Cereal Breeding Section, Morocco).

[d]Plant height and disease severity readings were measured at plant growth stage 10 on Feekes scale (20).

[e]Variety has not been released yet.

[f](-) indicates data not available.

Table 3. Mean differences between BYDV-inoculated and control barley varieties for disease severity index, plant height, biological and grain yield (numbers in parentheses represent the performance of the virus inoculated treatments as a percent of the controls)

Variety	Release date[a]	Relative crop importance[b]	Source of the seed[c]	Biological yield (g)	Grain yield (g)	Plant height (cm)[d]	Disease index (0-9)[d]
Arupo	1988	0	INRA	57(29)	20(5)	16(61)	6
Rihane 03	ny[e]	0	INRA	60(12)	17(4)	20(53)	7
Mona x Maris Dingo	ny	0	INRA	64(11)	11(15)	30(55)	6
Asni	1984	0	INRA	67(18)	24(15)	26(53)	5
Harmal	1988	0	INRA	78(15)	10(16)	38(36)	8
Arig 8	1961	2	INRA	85(23)	22(10)	29(49)	5
ACSAD 60	1984	1	INRA	86(18)	11(14)	25(57)	5
Matnan	ny	0	INRA	87(13)	29(5)	11(46)	6
Brasserie Maroc(895)	1973	3	INRA	90(8)	14(4)	21(55)	6
ER/APAM	1988	0	INRA	93(14)	24(9)	25(56)	6
Tamlalt	1984	0	INRA	97(6)	22(4)	24(54)	7
Tissa	1984	0	INRA	100(15)	16(4)	21(54)	7
ACSAD 68	1985	0	INRA	102(10)	29(7)	28(48)	6
ACSAD 176	1984	0	INRA	110(8)	34(2)	35(45)	6
Rabat (071)	1956	1	INRA	117(19)	36(9)	26(76)	6
Barlis(628)	1956	0	INRA	158(10)	42(5)	29(52)	6
Merzaga(071)	1956	2	INRA	171(13)	39(8)	27(56)	6
LSD 5%				36	10	9	-[f]
1%				49	14	13	-

[a]The release was made by the Moroccan Ministry of Agriculture and the Agrarian Reforme on dates indicated.

[b]Hectarage covered by the variety: 0 = 1 to 5%, 1 = 5.1 to 15%, 2 = 15.1 to 30%, and 3 = >30% in Morocco.

[c]The origin was INRA (National Agronomic Research Institute, Cereal Breeding Section, Morocco).

[d]Plant height and disease severity readings were measured at plant growth stage 10 on Feekes scale (20).

[e]Variety has not been released yet.

[f](-) indicates data not available.

Table 4. Mean differences between BYDV-inoculated and control oat varieties for disease severity index, plant height, biological and grain yield (numbers in parentheses represent the performance of the virus inoculated treatments as a percent of the controls)

Variety	Release date[a]	Relative crop importance[b]	Source of the seed[c]	Biological yield (g)	Grain yield (g)	Plant height (cm)[d]	Disease index (0-9)[d]
Albion	-[e]	0	U.Ill.	27(87)	7(87)	12(83)	3
Jaycee	1967	0	U.Ill.	38(77)	13(70)	16(71)	3
Golden	-	0	U.Ill.	63(37)	14(35)	23(56)	4
Clintland'64	-	0	U.Ill.	78(27)	43(7)	20(60)	7
Romani 153	-	3	INRA	127(15)	34(6)	40(49)	7
CI 5068	-	0	U.Ill.	135(53)	31(46)	12(80)	3
Saia	1973	0	U.Ill.	153(31)	35(31)	14(73)	3
Otee	1982	0	U.Ill.	168(26)	42(22)	25(64)	4
LSD 5%				99	25	9	-
1%				137	34	12	-

[a]The release of Romani 153 was made by the Moroccan Ministry of Agriculture and the Agrarian Reforme and by the Illinois State Department of Agriculture for the others, on dates indicated.

[b]Hectarage covered by the variety: 0 = 1 to 5%, 1 = 5.1 to 15%, 2 = 15.1 to 30%, and 3 = >30% in Morocco.

[c]The origin was University of Illinois (H. Jedlinski), or INRA (Agronomic Research Institute, Cereal Breeding Section, Morocco).

[d]Plant height and disease severity readings were measured at plant growth stage 10 on Feekes scale (20).

[e](-) indicates data not available.

Table 5. Effect of barley yellow dwarf virus on symptom development and head filling of the bread wheat varieties Nesma 149 and Saada

Treatment[a]	Parameters measured	
	No of diseased plants per meter of row[b]	Percent heads with blasted florets[c]
BYDV-inoculated	4.5	13.1
Uninoculated unsprayed check	2.7	4.7
Insecticide sprayed	1.8	2.2

[a]Plots receiving insecticide were treated with Deltamethrine every 3 weeks until heading. The virus treated plots were inoculated with the PAV strain of BYDV, using R. padi as a vector, at tillering stage.

[b]Diseased plants were counted at plant development stage 9-10 on Feekes scale (20).

[c]Percent heads with blasted florets were counted at stage 11 on Feekes scale (20).

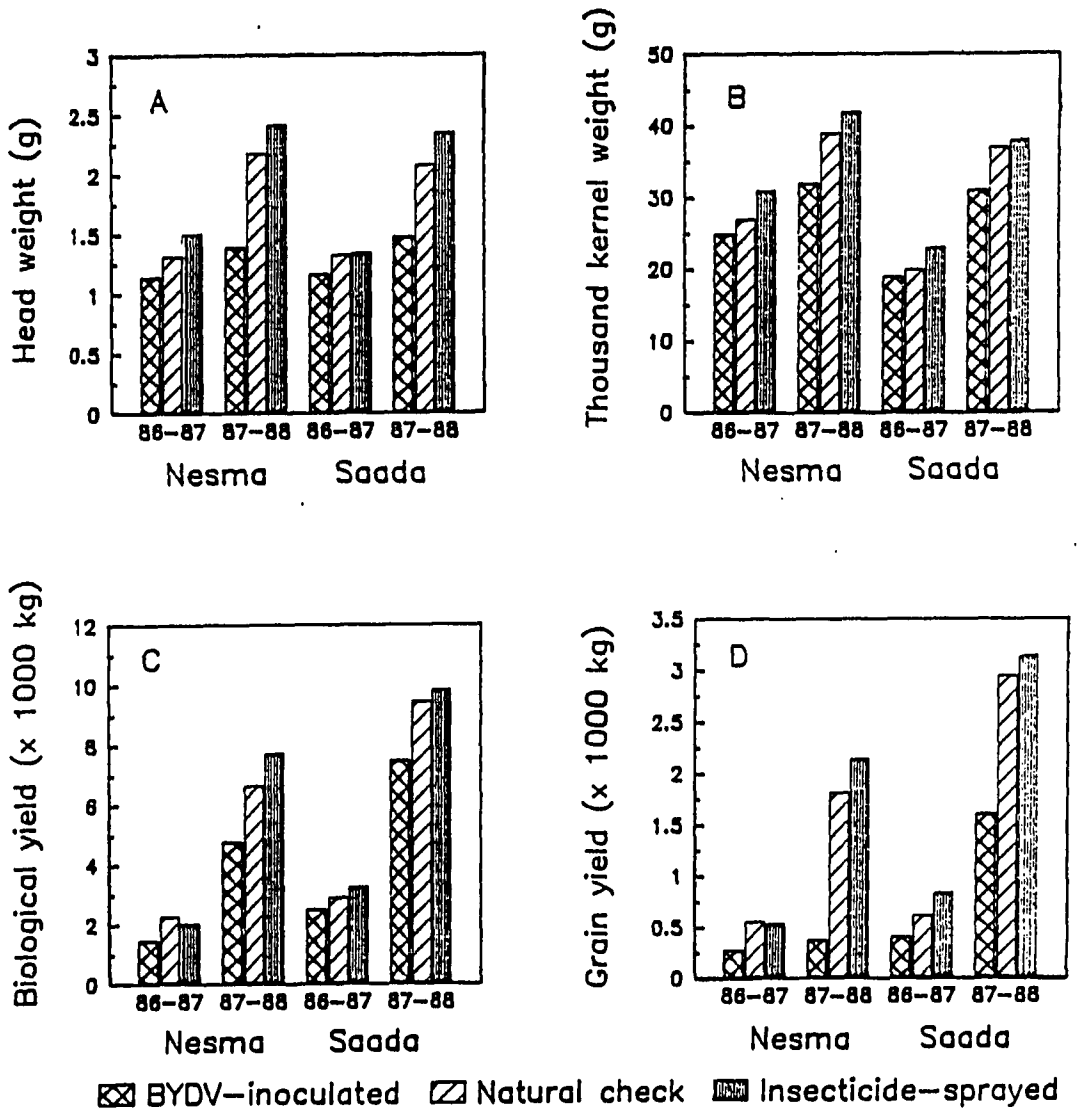
Table 6. Interaction between variety and virus treatments[a] for the size, biological and grain yields of cereal varieties from a split-plot design.

	Significance of the Interaction between virus treatments and varieties for		
	Plant height	Biological yield	Grain yield
Durum wheat	ns	**	*
Bread wheat	ns	**	ns
Barley	ns	**	**
Oat	**	**	**

[a]The 2 treatments used in the experiments were virus-inoculated and insecticide-protected hill plots.

** designates significant at P = 0.01, * designates significant at P = 0.05, ns = not significant.

Figure 1. Head weight (A), thousand kernel weight (B), biological (C) and grain yield (D) of two bread wheat varieties tested for 2 yr at three locations in West Central Morocco


A
Head weight (g)
3
2.5
2
1.5
1
0.5
0
86-87 87-88 86-87 87-88
Nesma Saada
B
Thousand kernel weight (g)
50
40
30
20
10
0
86-87 87-88 86-87 87-88
Nesma Saada
C
Biological yield (x 1000 kg)
12
10
8
6
4
2
0
86-87 87-88 86-87 87-88
Nesma Saada
D
Grain yield (x 1000 kg)
3.5
3
2.5
2
1.5
1
0.5
0
86-87 87-88 86-87 87-88
Nesma Saada
BYDV-inoculated
Natural check
Insecticide-sprayed

SUMMARY AND DISCUSSION

West-central Morocco represents a good part of the so-called semi-arid and arid regions of the country with only 200 to 400 mm annual rainfall. These regions cover 87% of arable lands and represent 59% of the hectarage sown to cereals in Morocco which produce 55% of the national production. However, yields of the main cereal crops in these areas have remained strikingly low. The most significant reasons for the poor yields include poor and erratic rainfall, marginal genetic potential of the material grown, the wide occurrence of Hessian fly in the area and the common and widespread fungal and viral diseases such as caused by BYDV. The objectives of this study have been to investigate the epidemiology of the disease, its importance in the area and consideration of some control options. Investigations have included virus strains present and their relative importance in the area, the vectors, the grass hosts, and the survival and maintenance of the virus-vector complex. Crop yield losses, germplasm screening for resistance to the virus and use of insecticide to control vectors were examined. Purification of the virus and preparation of an antiserum to one isolate of the virus were achieved. During the term of this study, interesting results were obtained which form the basis for conclusions and suggestions presented herein. We hope these conclusions and suggestions will be beneficial to the agricultural income both in the aridoculture area, in particular, and in the country.

The first important result of this study was the excellent agreement between ELISA and aphid transmission for virus strain identification in Morocco. This conclusion permitted the use of a specific and rapid

serological test to screen for the virus isolates present in the area instead of the laborious and time consuming aphid transmission technique. Results concerning relative spread and occurrence of virus strains using field surveys and trap plants revealed an abundance of the vector-non-specific (PAV) strain. This was followed by the Sitobion avenae-specific (MAV), and the Rhopalosiphum padi-specific (RPV) strains. Specific reagents to test for the other known strains such as the R. maidis-specific (RMV) and Schizaphis graminum-specific (SGV) are still unavailable in Morocco. Thus, information regarding their relative importance in the area is unavailable. The occurrence of the RMV strain in Morocco was reported, however, in one of our previous reports (17, 18). The predominance of the PAV strain constitutes a threat to the cereal crops in the region since it represents the most virulent strain, and is known to be vectored nonspecifically by many grass aphid species. This strain was purified and a specific antiserum was prepared against it. Consequently, this will provide local immunoreagents specific to this strain and make possible additional surveys in other parts of the country. Furthermore, it will assist breeders by providing rapid methods for preliminary resistance screening. In addition, the potential similarity of the PAV and MAV strains identified in this study to their European and American counterparts was discussed. Study of the virus vectors increased the number of known virus vectors in the country to ten. Some of these species have been, to the best of our knowledge, tested only in Morocco. R. padi appeared to be the most abundant and efficient vector responsible for virus transmission and maintenance in the area. R. maidis and S. graminum

demonstrated higher transmission efficiency than that usually associated with the PAV strain. Of significant importance, was the occurrence of the aphid Diuraphis noxia, as an active virus vector in the area. The aphid species, Melanaphis donacis, collected on Arundo plants, used as hedges and wind breakers in the area, failed to vector the PAV strain in controlled transmission tests and also tested negative with antisera specific to MAV and RPV strains of the virus. The high number and diversity of the aphid species that proved to be vectors of the virus enhances the threat that BYD represents to the cereals grown in the area. At the same time, more research is needed to clarify the potential vector status of at least five other aphid species known to occur on grasses, including cereals, in the country but which were not tested in this study. Also when virus cultures of all the known endemic BYDV strains become available, it is advisable to assess all putative aphid vectors for their vector competence. This information will be valuable because mixed infections, commonly encountered in nature, can allow for vectoring of various vector-specific strains by otherwise nonvector aphid species.

This study suggested that the aphid species responsible for primary spread of the virus from its primary foci was R. padi, as demonstrated by the use of trap plants at different sites during 19 months. It also demonstrated that secondary spread occurred during early spring which coincided with arrival and maximum activity of other aphid species. These observations provide an explanation for the periods of maximum disease incidence during the spring months observed in this study.

The diversity of grass species that maintain the virus inoculum and can support vector colonies add complexity to the disease cycle in Morocco. Sixteen different grasses that were collected in the area and tested serologically for virus presence were susceptible. In addition, results from artificial inoculation tests involving 17 new grass introductions for pasture improvement demonstrated that 10 were susceptible with external symptoms, 5 were symptomless carriers, and only two were immune. Most of these local as well as introduced grasses were either annual or perennial species. This situation favors maintenance of the virus-vector complex in the country. The role that maize, as an oversummering host for the virus-vector complex and the implication that cereal volunteers, including the cereal-legume mixtures, plays to provide alternate hosts during the period between maize and cereal crops was demonstrated. These findings imply that sound crop management techniques such as the use of grass herbicides, destruction of maize residues, proper setting of harvesting machinery to minimize lost seed and therefore volunteers, tillage at appropriate time to destroy volunteers at emergence, search for other forage resources to replace legume-cereal mixtures under irrigation, and the synchronization of these management techniques among the farmers will alleviate the threat of BYD epidemics in the area.

An epiphytotic of BYD occurred during the 1986-87 growing season. Climatic conditions of this season and those of the preceding ones together with the accumulated information on inoculum maintenance, vectors and grass hosts may constitute initial data to model and predict the disease incidence and severity in the future. Additional information must be

obtained concerning vector population dynamics in the region. For this purpose, consideration should be given to joining the European network EURAPHID.

Quantification of yield loss was measured by using the bread wheat varieties Nesma 149 and Saada. Plots artificially inoculated with BYDV-PAV, protected with an insecticide (Deltamethrine 2.5% a.i.), or left untreated and uninoculated were examined for two consecutive growing seasons at three sites in the aridoculture area. Some interaction between the virus treatments, years, and varieties was observed for some of the parameters measured. Artificial inoculation with virus induced the highest number of diseased plants and heads with blasted terminal florets. This treatment also induced highly significant losses compared to the insecticide protected control. These were principally a 19 to 38% decrease in head weight, 9 to 25% decrease in thousand kernel weight, a 38% to 24 % decrease in biological yield and a 61% to 51% decrease in the grain yields of the cultivars Nesma 149 and Saada, respectively. Losses resulting from naturally occurring virus infection, as compared with the insecticide treated control, were 10% for the head weight, 11% for the thousand kernel weight, 14% for the Nesma 149 biological yield, 11% for the Saada biological yield, and 15% and 26% for Nesma 149 and Saada grain yield, respectfully. The yield loss data, in general, paralleled the conclusions made from examination of field survey results made during the 1986-87 growing season which suggested very high BYD disease incidence.

Comparison of data from the 2 varieties, suggested Saada was more susceptible to BYDV than Nesma 149. However, Nesma 149 appeared to have

some field tolerance under heavy virus incidence. It may be instructive to investigate more closely the response of this variety to virus inoculation at different growth stages and compare results with those found by Comeau (11).

Assessment of varietal resistance to BYDV included analysis of 8 durum and 19 bread wheats, 17 barley and 8 oat cultivars. The bread wheats included one spring and 2 winter cultivars introduced from Indiana. Evaluation of virus infection on plant height, total biomass, grain yield and intensity of color change (disease severity index) of all entries grown in hill plots suggested that the bread wheats were more tolerant to the virus, followed by durum wheats and barleys which showed no appreciable virus resistance. Some oat varieties obtained from Illinois had good tolerance to the virus. The spring bread wheats ACSAD 59, Jouda, BT.J15 and BT.J16 and the spring durum wheats Cocorit, ACSAD 65, and Marzak showed some tolerance to BYDV. The two winter bread wheats Caldwell and Elmo showed very little color change following inoculation and therefore may be good cultivars for use at high elevations in Morocco or for sources of resistance genes to incorporate in well adapted spring types. The other introduced spring wheat, Laudrina, was susceptibile to the Moroccan virus isolate. Saada, the newly identified Hessian fly resistant variety, was highly susceptible to BYDV. If the cultivar Saada is to be widely promoted in the area because of the damaging level of Hessian fly in the region, important yield losses due to BYDV are to be expected. Tolerant oats such as Albion, Jaycee, and Golden may be good substitutes for the highly susceptible Romani 153 which currently is the only variety grown in the

country. These varieties can also be grown in pure stands or in forage mixtures with legumes to replace the susceptible materials presently used. Finally, no barley cultivars tested showed appreciable resistance to the virus for any parameters evaluated, with the exception of Harmal, ACSAD 60, and Mona x Maris Dingo which yielded somewhat better than the other cultivars when infected with BYDV. The extreme earliness of the Mona x Maris Dingo makes adoption by the growers unattractive. Consequently, serious efforts should be made to improve resistance of barley cultivars in the area because of the vital role this species still plays in agriculture of the region. This should be readily accomplished since resistance in barley is controlled by a major Yd_2 gene that can be easily backcrossed into well-adapted local genotypes. While testing this collection of germplasm, lines were tested under high inoculum pressure and some forcing of the virus and the vector on all entries may not totally reflect naturally occurring events.

Finally, I cannot convince myself to close this discussion without alluding to the fact that the BYD disease is an intricate subject for which results and conclusions are, for most of the time, hard to generalize and extrapolate to numerous other geographical or ecological areas. Therefore, unless research on various aspects of the disease is done "in situ", other findings may only be applicable or transferable with potential for a high risk of failure.

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APPENDIX I. COMPARISON OF NORTH AMERICAN (U.S.A.) WITH EUROPEAN ANTIBODIES IN REACTIONS WITH MOROCCAN ISOLATES OF BYDV

Antisera compared[a]	Degrees of Freedom	X^2 values	Probability
F vs 2B12	1	64.14	< 0.01
B vs 3A11	1	87.52	< 0.01

[a]The antisera F and B are acquired from Europe (BIOREBA, Switzerland) and are known to be specific to MAV and PAV European strains, respectively. The antisera 2B12 and 3A11 are U.S.A. counterparts prepared at Iowa State University.

APPENDIX II. AVERAGE WIND SPEED DETERMINED AT DIFFERENT WEATHER STATIONS ACROSS MOROCCO (ORIGINAL DATA - DAILY READINGS AT 18:00 HR)[a]

Weather station and (number of years)		Wind speed averages calculated per station and per month on the basis ofthe total number of years shown in parentheses.											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Ouarza-zate (36)	V[b]	2.8	4.7	6.7	7.9	7.1	7.2	7.0	6.7	5.5	4.5	3.1	2.8
	SD	1.1	1.5	1.8	1.5	1.7	1.5	1.4	1.2	1.3	1.6	1.1	1.2
Kenitra (35)	V	2.9	3.9	5.0	5.5	5.0	5.7	5.3	5.2	4.9	3.8	2.8	2.7
	SD	0.8	0.9	0.9	0.8	1.1	0.8	1.0	1.0	0.8	0.7	0.8	0.9
Alho-ceima (22)	V	1.8	2.5	2.9	2.7	2.2	3.1	2.8	2.8	2.7	2.3	1.8	1.7
	SD	0.6	0.9	1.1	0.6	0.5	1.0	0.7	0.9	0.8	0.8	0.5	0.7
Midelt (35)	V	5.2	6.5	7.6	7.6	7.2	6.5	5.9	6.0	5.8	5.3	4.7	5.0
	SD	1.4	1.5	1.5	1.4	1.4 4	1.1	1.0	1.1	0.9	1.3	1.2	1.5
Tetouan (25)	V	4.8	5.2	5.3	5.3	5.6	5.8	5.6	5.3	5.1	4.6	4.2	4.6
	SD	1.2	1.1	1.3	1.1	1.	1.3	1.4	1.3	1.1	1.2	1.3	0.9
Safi (31)	V	3.2	4.1	4.6	5.3	5.3	5.4	5.9	5.5	4.9	4.3	3.5	3.2
	SD	1.0	1.0	0.8	1.1	1.4	1.4	1.3	1.2	1.3	1.0	0.9	0.8
Laayoune (8)	V	22.1	16.7	19.4	16.7	15.8	15.7	16.6	16.0	15.5	14.9	16.4	16.6
	SD	7.7	5.5	4.2	2.9	3.8	1.7	1.6	3.6	3.0	3.0	4.7	3.7
Errachi-dia (13)	V	2.6	3.5	4.3	5.1	5.1	5.6	5.4	5.1	4.5	3.2	2.4	2.3
	SD	0.7	0.8	0.6	0.7	0.6	0.8	0.9	0.8	0.5	0.7	0.6	0.8

[a]Original data were obtained from the National Meteorological Center, Casablanca, Morocco.

[b]The symbol V stands for wind speed in m/s and SD for the standard deviation for respective stations and months.

APPENDIX III. WIND DIRECTION FREQUENCIES AVERAGED FOR DATA[a] RECORDED OVER A PERIOD OF 8 TO 34 YEARS AT EIGHT DIFFERENT WEATHER STATIONS ACROSS MOROCCO

Wind direction frequency averages (percent) determined monthly.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
E[b]	5.5	6.8	8.8	12.3	7.6	8.8	10.5	7.2	10.8	9.4	7.2	7.3
NE	4.7	7.9	5.3	4.8	7.1	10.0	6.3	15.9	9.6	8.6	7.1	3.9
N	16.2	13.4	20.5	24.4	24.9	0.7	22.0	25.8	27.8	23.1	13.9	22.4
NW	17.0	7.4	9.5	6.4	15.0	9.9	7.9	5.6	5.0	5.0	9.3	8.9
W	28.3	39.9	34.3	35.6	29.2	22.5	21.4	23.0	26.2	33.2	34.3	24.1
SW	18.7	9.2	14.6	8.4	12.1	10.6	9.5	11.6	6.1	13.0	14.6	21.2
S	7.6	9.2	7.0	8.0	5.1	5.5	4.6	10.9	10.3	7.7	9.8	12.2
SE	1.8	6.1	-[c]	-	-	3.2	1.8	-	4.2	-	3.8	-

[a]Raw data were obtained from the national meteorological center, Casablanca, Morocco.

[b]The direction symbols used represent east = E, northeast = NE, north = N, northwest = NW, west = W, southwest = SW, south = S, and southeast = SE.

[c]The minus sign indicates the direction was not recorded for those months.

APPENDIX IV. MEAN MONTHLY TEMPERATURE (C) AND PRECIPITATION (MM) RECORDED AT THREE LOCATIONS IN WEST-CENTRAL MOROCCO FOR THE 1986-87 AND 1987-88 GROWING SEASONS

	Location and growing season											
	Sidi El Aidi				Jemmaa Shaim				Tessaout			
Month	86-87		87-88		86-87		87-88		86-87		87-88	
	T[a]	P[a]	T	P	T	P	T	P	T	P	T	P
Sep.	21.8	0.0	25.5	13.4	22.3	0.0	26.7	8.0	24.4	0.0	27.15	35.8
Oct.	19.5	0.0	19.6	29.3	19.7	0.0	17.2	70.5	18.5	34.0	19.3	13.6
Nov.	15.7	38.5	15.3	62.2	15.0	32.5	11.9	60.5	14.7	34.6	15.9	34.6
Dec.	12.0	5.0	14.9	140.8	12.0	9.7	11.7	91.5	10.8	0.2	12.5	53.5
Jan.	13.8	45.0	11.5	113.6	13.0	51.0	9.4	126.0	12.6	34.0	9.9	63.7
Feb.	13.4	57.0	12.1	73.5	14.2	63.6	12.1	44.2	12.1	63.1	11.9	32.0
Mar.	15.5	38.1	14.3	14.8	18.4	10.5	19.5	32.5	15.7	13.5	14.8	39.4
Apr.	18.9	12.8	15.5	5.9	19.8	16.5	20.8	0.0	19.8	0.7	16.8	3.1
May	17.8	7.0	17.6	16.7	22.8	15.0	18.2	14.0	19.2	0.0	14.2	2.5
Total		203.4		470.2		198.8		447.2		180.1		278.2

[a]T represents temperature, and P represents precipitations.

APPENDIX V. ANALYSIS OF VARIANCE ON THE EFFECTS OF YEAR, LOCATION, VARIETY, AND BYDV TREATMENT[a] ON THE NUMBER OF DISEASED PLANTS/METER OF ROW, PERCENT HEADS WITH BLASTED FLORETS, YIELD COMPONENTS, AND YIELD OF TWO VARIETIES STUDIED IN A SPLIT-PLOT DESIGN WITH THREE REPLICATIONS

		Mean Square					
Source of variation	D.F.	Diseased plants/ meter of row	Percent heads w/ blasted florets	Head weight	Thousand kernel weight	Biological yield	Grain yield
Year(Y)	1	55.9**	19.6	12.4	4097.4**	74208.9**	6417.5**
Location (L)	2	7.7	191.5**	1.1**	235.3**	9216.3**	1826.9**
Y * L	2	42.1**	182.5**	1.1**	294.6**	3235.0**	582.0**
Error (a)	12	4.5	14.4	0.1	5.7	144.7	11.5
Variety (V)	1	13.3	29.1	0.0	617.5**	8334.0**	867.0**
V * Y	1	15.5	116.7	0.0	113.7**	1707.6**	436.6**
V * L	2	3.2	109.8*	0.1	4.1	198.2	21.6*
V * Y * L	2	0.2	31.7	0.6**	231.4**	563.5*	11.9
Error (b)	12	3.4	20.8	1.1	5:3	99.2	4.2
Virus							
Treatments(T)[a]	2	788.4**	1167.9**	5.5**	374.1**	2667.6**	782.3**
NT vs INS	1	50.3**	109.9**	0.5**	109.4**	294.0**	56.9**
BYDV vs (NT+INS)	1	1526.9**	2225.9**	6.5**	638.8**	5041.3**	1507.7**

[a]The three treatments included in this study were the insecticide sprayed (INS), the barley yellow dwarf virus inoculated (BYDV), and the uninoculated unsprayed (NT) plots repeated three times for two varieties, Nesma 149 and Saada in a split-plot design for two years at three locations.

* Designates significant at P = 0.05; ** designates significant at P = 0.01.

APPENDIX V. (continued)

Source of variation	D.F.	Mean Square					
		Diseased plants/ meter of row	Percent heads w/ blasted florets	Head weight	Thousand kernel weight	Biological yield	Grain yield
T * Y	2	148.5**	102.5**	1.1**	57.6**	917.2**	304.2**
Y * NT vs INS	1			0.1	3.5		
Y * BYDV vs (NT+INS)	1			2.2**	111.8**		
T * L	4	5.8	34.8**	0.0	37.0**	165.9**	23.6**
T * Y * L	4	23.6**	140.1**	0.0	10.1	84.1	7.7
T * V	2	23.7**	5.2	0.1	24.0**	10.9	9.3
T * V * Y	2	14.9*	16.4	0.0	1.8	100.1*	11.7*
V * Y * NT vs INS	1					198.0*	15.6*
V * Y * BYDV vs (NT+INS)	1					2.3	7.7
T * V * L	4	1.8	14.0	0.1	9.8	22.4	3.2
T * V * Y * L	4	0.5	31.7**	0.2**	10.8**	85.4	13.1**
Error (c)	48	3.9	6.1	0.1	4.1	37.7	3.2

APPENDIX VI. EFFECT OF BARLEY YELLOW DWARF VIRUS ON THE HEAD WEIGHT OF TWO BREAD WHEAT VARIETIES EVALUATED OVER TWO YEARS AT THREE LOCATIONS USING A SPLIT-PLOT DESIGN

	Average head weight (g) of the indicated varieties			
	Nesma 149		Saada	
Treatments[a]	1986-87	1987-88	1986-87	1987-88
BYDV-inoculated	1.2	1.4	1.2	1.5
Uninoculated unsprayed check	1.3	2.2	1.3	2.1
Insecticide sprayed	1.5	2.4	1.4	2.4

[a]Plots receiving insecticide were treated with Deltamethrine every 3 weeks until heading. The virus treated plots were inoculated with the PAV strain of BYDV using Rhopalosiphum padi as a vector at tillering stage.

APPENDIX VII. EFFECT OF BARLEY YELLOW DWARF VIRUS ON THE THOUSAND KERNEL WEIGHT OF TWO BREAD WHEAT VARIETIES EVALUATED OVER TWO YEARS AT THREE LOCATIONS USING A SPLIT-PLOT DESIGN

	Average thousand kernel weight (g) of the indicated varieties			
	Nesma 149		Saada	
Treatments[a]	1986-87	1987-88	1986-87	1987-88
BYDV-inoculated	25.0	32.0	19.0	31.0
Uninoculated unsprayed check	27.0	39.0	20.0	37.0
Insecticide sprayed	31.0	42.0	23.0	38.0

[a]Plots receiving insecticide were treated with Deltamethrine every 3 weeks until heading. The virus-treated plots were inoculated with the PAV strain of BYDV using Rhopalosiphum padi as a vector at tillering stage.

APPENDIX VIII. EFFECT OF BARLEY YELLOW DWARF VIRUS ON THE BIOLOGICAL YIELD OF TWO BREAD WHEAT VARIETIES EVALUATED OVER TWO YEARS AT THREE LOCATIONS USING A SPLIT-PLOT DESIGN

	Average biological yields(x 1000 kg) of the plots sown to the indicated varieties			
	Nesma 149		Saada	
Treatments[a]	1986-87	1987-88	1986-87	1987-88
BYDV-inoculated	1.5	4.8	2.5	7.5
Uninoculated unsprayed check	2.3	6.7	2.9	9.5
Insecticide-sprayed	2.1	7.8	3.3	9.9

[a]Plots receiving insecticide were treated with Deltamethrine every 3 weeks until heading. The virus treated plots were inoculated with the PAV strain of BYDV using Rhopalosiphum padi as a vector at tillering stage.

APPENDIX IX. EFFECT OF BARLEY YELLOW DWARF VIRUS ON THE GRAIN YIELD OF TWO BREAD WHEAT VARIETIES EVALUATED OVER TWO YEARS AT THREE LOCATIONS USING A SPLIT-PLOT DESIGN

	Average grain yields(x 1000 kg) of the plots sown to the indicated varieties			
	Nesma 149		Saada	
Treatments[a]	1986-87	1987-88	1986-87	1987-88
BYDV-inoculated	0.3	0.4	0.4	1.6
Uninoculated unsprayed check	0.6	1.8	0.6	2.9
Insecticide sprayed	0.5	2.2	0.8	3.1

[a]Plots receiving insecticide were treated with Deltamethrine every 3 weeks until heading. The virus treated plots were inoculated with the PAV strain of BYDV using Rhopalosiphum padi as a vector at tillering stage.

APPENDIX X. ANALYSIS OF VARIANCE ON THE EFFECT OF BYDV ON THE SIZE, DISEASE INDEX, BIOLOGICAL AND GRAIN YIELDS OF CEREAL VARIETIES FROM A SPLIT-PLOT DESIGN

Source of variation	Degrees of freedom	Mean square: Plant height (cm)	Disease Index (0-9)	Biol. yield (g)	Grain yield (g)
Durum wheat varieties:					
Variety (V)	7	241.9 **	3.9 **	3465.8 **	266.4 **
Error a (= Rep*V)	14	18.0	0.7	337.4	26.4
BYDV					
Inoculation (I)	1	5482.7 **		71688.0 **	3976.1 **
V*I	7	57.2 ns		2794.5 **	100.7 *
Error b (= Rep*V*I)	16	32.5		473.7	27.8
Bread wheat varieties:					
Variety (V)	16	316.3 **	4.7 **	374.0 ns	18.9 ns
Error a (= Rep*V)	32	28.9	0.5	590.1	20.2
BYDV					
Inoculation (I)	1	12607.4 **		109499.6 **	4241.4 **
V*I	16	51.2 ns		578.2 **	20.5 ns
Error b (= Rep*V*I)	34	38.3		537.0	22.4

** Designates significant at P = 0.01; * designates significant at P = 0.05, ns = not significant.

APPENDIX X. (continued)

Source of variation	Degrees of freedom	Mean square: Plant height (cm)	Disease Index (0-9)	Biol. yield (g)	Grain yield (g)
Barley varieties:					
Variety (V)	16	169.9 **	2.0 **	2426.2 **	224.6 **
Error a (= Rep*V)	32	30.9	0.7	368.1	39.8
BYDV					
Inoculation (I)	1	16901.7 **		232136.5 **	13617.0 **
V*I	16	62.9 ns		1470.1 **	167.1 **
Error b (= Rep*V*I)	34	34.1		477.4	38.7
Oat varieties:					
Variety (V)	7	332.8 **	8.2 **	18488.1 **	550.6 *
Error a (= Rep*V)	14	39.3	0.5	4498.1	233.9
BYDV					
Inoculation (I)	1	4900.5 **		117018.7 **	8893.6 **
V*I	7	126.7 **		4378.3 **	302.4 **
Error b (= Rep*V*I)	16	26.4		3314.6	211.0